ABSTRACT

This study investigated the role of ionotropic and metabotropic glutamate receptors in the deficits in brain reward function, as measured by elevations in intracranial self-stimulation (ICSS) reward thresholds, associated with nicotine withdrawal. The group II metabotropic glutamate (mGluII) receptor agonist LY314582 (a racemic mixture of LY354740 [\(+\)-2-amino-2-[1S,2S]-carboxycyclopropan-1-yl]-3-[xanth-9-yl]propionic acid] (2.5–7.5 mg/kg) precipitated withdrawal-like elevations in ICSS thresholds, a sensitive measure of reward function, in nicotine-dependent but not control rats. LY314582 did not affect response latencies, a measure of performance in the ICSS paradigm. Bilateral microinfusion of LY314582 (10–100 ng/side) into the ventral tegmental area likewise precipitated dose-dependent threshold elevations in nicotine-dependent rats. Furthermore, a single injection of the mGluII receptor antagonist LY341495 (2S-2-amino-2-[1S,2S]-carboxycyclopropan-1-yl]-3-[xanth-9-yl]propionic acid) (1 mg/kg) attenuated the threshold elevations observed in rats undergoing spontaneous nicotine withdrawal. mGluII receptors are primarily located on glutamatergic terminals throughout the mesocorticolimbic system, where they act as inhibitory autoreceptors. To investigate whether mGluII receptors contributed to nicotine withdrawal by decreasing glutamatergic transmission, we next examined whether direct blockade of postsynaptic glutamate receptors precipitated withdrawal-like reward deficits in nicotine-dependent rats. The \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoylbenzof\(\phi\)quinoxaline (NBQX; 0.01–1 mg/kg) precipitated withdrawal-like threshold elevations in nicotine-dependent but not control rats, whereas 6-methyl-2-[phenylethynyl]-pyridine (MPEP; 0.01–3 mg/kg) and dizocilpine (MK-801; 0.01–0.2 mg/kg), antagonists at metabotropic glutamate 5 and N-methyl-\(\phi\)-aspartate receptors, respectively, did not. Overall, these data demonstrate that mGluII receptors play an important role in the reward deficits associated with nicotine withdrawal. Furthermore, it is likely that mGluII receptors generate this reward deficit, at least in part, by decreasing glutamate transmission at AMPA/kainate receptors.

There is now compelling evidence that the aversive withdrawal syndrome observed during periods of nicotine abstinence contributes to the persistence of the tobacco habit in smokers (Hughes, 1992; Kenny and Markou, 2001). Nicotine withdrawal was shown to precipitate a deficit in brain reward function, as measured by elevations in intracranial self-stimulation (ICSS) reward thresholds, similar to that observed in rats undergoing withdrawal from other major drugs of abuse (Epping-Jordan et al., 1998). Moreover, avoidance and alleviation of this deficit in brain reward function has been proposed as a major motivational factor contributing to craving, relapse, and continued tobacco consumption in human smokers (Epping-Jordan et al., 1998; Kenny and Markou, 2001). In contrast to the intense investigations into the mechanisms by which acute nicotine produces its rewarding effects, little is known concerning the mechanisms mediating the reward deficits associated with nicotine withdrawal.

Most drugs of abuse have been shown to stimulate excitatory glutamatergic transmission throughout brain reward circuitries (Kalivas and Duffy, 1998; Wolf et al., 2000). Increases in glutamatergic transmission have been shown to play an important role in mediating the positive reinforcing actions of addictive drugs (Harris and Aston-Jones, 2003).

ABBREVIATIONS: ICSS, intracranial self-stimulation; VTA, ventral tegmental area; mGluII, group II metabotropic glutamate receptor; NMDA, \(\text{N}-\text{methyl-}\phi-\text{aspartate; AMPA, } \alpha\text{-amino-3-hydroxy-5-methyl-4-isoxazole propionate; mGlu5, metabotropic glutamate 5 receptors; MK-801, dizocilpine; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzof}\(\phi\)quinoxaline; MPEP, 6-methyl-2-[phenylethynyl]-pyridine; ANOVA, analysis of variance.
Indeed, it is thought that nicotine elicits its rewarding actions, at least in part, by activating nicotinic acetylcholine receptors located on glutamate terminals in the ventral tegmental area (VTA), thereby potentiating excitatory glutamatergic transmission in this reward-relevant brain site and increasing mesoaccumbens dopamine transmission (Mansvelder and McGehee, 2000). Accordingly, blockade of glutamatergic transmission reduced nicotine’s stimulatory action on mesoaccumbens dopamine transmission (Schilstrom et al., 1998) and attenuated the rewarding actions of nicotine and other drugs of abuse (Chiamulera et al., 2001; Laviolette and van der Kooy, 2003; Paterson et al., 2003).

It has been suggested that the neuroadaptations that occur during prolonged exposure to drugs of abuse, which give rise to the deficits in brain reward function associated with withdrawal, may reside in the same neural elements that mediate the acute rewarding actions of these drugs (Koob and Le Moal, 2001). Indeed, in contrast to nicotine’s acute stimulatory effects, nicotine withdrawal attenuated mesoaccumbens dopamine transmission (Hildebrand et al., 1997), an action likely to contribute to the reward and motivational deficits associated with nicotine withdrawal (Kenny and Markou, 2001). Therefore, because increases in excitatory glutamatergic transmission are believed to play an important role in the reinforcing actions of acute nicotine, we hypothesized that withdrawal from nicotine is associated with decreased glutamatergic transmission in brain reward circuitries, which contributes to the reward deficits observed during withdrawal. To test this hypothesis, the effects of a group II metabotropic glutamate (mGluII) receptor agonist were examined in nicotine-treated and control rats. mGluII receptors, comprising of mGlu2 and mGlu3 receptors, are inhibitory autoreceptors located on glutamate terminals throughout the mesocorticolimbic system, where they act to decrease excitatory glutamatergic transmission (Bonci et al., 1997; Wigmore and Lacey, 1998). Because mGluII receptor agonists decrease glutamatergic transmission in brain reward circuitries (Manson and Williams, 1999), we predicted that activation of these receptors would precipitate ICSS threshold elevations in nicotine-dependent rats similar to those observed in rats during spontaneous nicotine withdrawal, whereas blockade of these receptors would reverse the threshold elevations associated with spontaneous nicotine withdrawal. To further investigate the role of glutamatergic transmission in nicotine withdrawal, we also examined whether direct blockade of glutamatergic transmission at postsynaptic NMDA, AMPA/kainate, and metabotropic glutamate 5 (mGlu5) receptors precipitated withdrawal-like ICSS threshold elevations in nicotine-dependent rats.

**Materials and Methods**

**Animal Housing.** Subjects were 149 male Wistar rats weighing 300 to 320 g at the start of each experiment. Rats were obtained from Charles River Laboratories (Raleigh, NC) and were housed in groups of two or three per cage, with food and water available ad libitum. Animals were maintained in a temperature-controlled vivarium under a 12-h light/dark cycle (lights off at 10:00 AM). Animals were tested during the dark portion of the light/dark cycle, except for the spontaneous nicotine withdrawal experiment when rats were tested at time points according to the experimental design. All animals were treated in accordance with the guidelines of the National Institutes of Health regarding the principles of animal care. Animal facilities and experimental protocols were in accordance with the Association for the Assessment and Accreditation of Laboratory Animal Care.

**Drugs.** (−)-Nicotine hydrocarbon tartrate salt [(−)-1-methyl-2-(3-pyridyl) pyrrolidine] and dizocilpine ([+]-MK-801) were purchased from Sigma-Aldrich (St. Louis, MO). LY341495 (2S-2-amino-2-[15S,2S-2-carboxyloxypropan-1-yl]-3-[xanth-9-yl]propionic acid) and NBQX disodium (2,3-dihydroxy-6-nitro-7-sulfamoylbenzof[1,4]oxazine disodium) were purchased from Tocris Cookson (Ballwin, MO). LY314582 (the racemic mixture of LY354740 [(+/-)-2-aminoisobutyric acid(3.1.0)hexane-2,6-dicarboxylic acid]) and 6-methyl-2-[phenylethyl]-pyridine (MPEP) were synthesized by one of the coauthors (F. Gasparini). Drugs were prepared immediately before each administration. For systemic administration, all drugs were dissolved in sterile water and administered by intraperitoneal injection, in a volume of 1 ml/kg body weight, 30 min before the experimental session. For direct intra-VTA administration, LY314582 was dissolved in artificial cerebrospinal fluid of the following composition: 126.6 mM NaCl, 27.4 mM NaHCO3, 2.4 mM KCl, 0.5 mM KH2PO4, 0.89 mM CaCl2, 0.8 mM MgCl2, 0.48 mM Na2HPO4 and 7.1 mM glucose, pH 7.4. Rats received intra-VTA injections immediately before the initiation of the experimental session. Unless otherwise stated, drug doses refer to the salt form.

**Apparatus.** Intracranial self-stimulation training and testing took place in 16 Plexiglas operant chambers (25 × 31 × 24 cm) (MED Associates, St. Albans, VT). The floors of the operant chambers were constructed of parallel aluminum rods spaced 1.25 cm apart. One wall contained a metal wheel manipulandum that required 0.2 N force to rotate it one-quarter of a turn. The wheel (5 cm in width) extended out of the wall – 3 cm. Each testing chamber was enclosed within a light- and sound-attenuated chamber (62 × 63 × 43 cm). Intracranial stimulation was delivered by constant current stimulators (Stimtech model 1200; San Diego Instruments, San Diego, CA). Subjects were connected to the stimulation circuit through flexible bipolar leads (Plastics One, Roanoke, VA) attached to gold-contact swivel commutators (model SL2C; Plastics One) mounted above the chamber. The stimulation parameters, data collection, and all test session functions were controlled by a microcomputer.

**Placement of Electrodes and Cannulas.** Rats were anesthetized by inhalation of 1 to 3% halothane in oxygen and positioned in a stereotaxic frame (Kopf Instruments, Tujunga, CA). The incisor bar was adjusted to 5 mm above the interaural line, and the skull exposed. Stainless steel bipolar electrodes (11 mm in length) were implanted into the posterior lateral hypothalamus (AP −5.5 mm; ML ±1.7 mm; DV 5.3 mm from skull surface; angle of 10° from midline), according to the atlas of Pellegrino et al. (1979). For the VTA infusion experiment, bilateral stainless steel guide cannulas (23-gauge, 14 mm in length) were implanted 3 mm above the VTA (AP −3.2 mm from bregma; ML ±1.7 mm; DV 5.3 mm from skull surface; angle of 10° from midline), at the same time that ICSS electrodes were implanted. Four indurations were made in the skull to accommodate screws that together with the application of dental acrylic, held the electrode and cannulas in place. Cannulas were kept patent using 14-mm-long stainless steel stylets (30-gauge). Animals were allowed to recover from surgery for at least 7 days before training in the ICSS paradigm.

**Osmotic Mini-Pump Surgery.** Rats were anesthetized by inhalation of 1 to 3% halothane in oxygen and prepared with Alzet osmotic mini-pumps [model 2ML4 (28 day); Alza, Palo Alto, CA] placed subcutaneously (back of the animal parallel to the spine). Pumps were filled with either sterile water or nicotine salt solution. The concentration of the nicotine salt solution was adjusted according to animal body weight, resulting in delivery of 9 mg/kg/day (3.16 mg/kg, free base). This dose of nicotine maintains stable plasma levels (~44 ng/ml) comparable with those obtained in human smokers consuming approximately 30 cigarettes per day (Benowitz, 1988). After mini-pump implantation (or removal), the surgical wound was
closed with 9-mm stainless steel wound clips (BD Biosciences Primary Care Diagnostics, Sparks, MD) and treated with topical antibiotic (Bacitracin) ointment.

ICSS Reward Threshold Procedure. Animals were trained to respond according to a modification of the discrete-trial current-threshold procedure of Kornetsky and Esposito (1979). Briefly, a trial was initiated by the delivery of a noncontingent electrical stimulus. This electrical reinforcer had a train duration of 500 ms and consisted of 0.1-ms rectangular cathodal pulses that were delivered at a frequency of 50 to 100 Hz. The frequency of the stimulation was selected for individual animals so that current-intensity thresholds of each subject were within 85 to 160 μA, and thus allowed both threshold elevations and lowerings to be detected. This frequency was held constant throughout the experiment. A one-quarter turn of the wheel manipulandum within 7.5 s of the delivery of the noncontingent electrical stimulation resulted in the delivery of an electrical stimulus identical in all parameters to the noncontingent stimulus that initiated the trial. After a variable intertrial interval (7.5–12.5 s), another trial was initiated with the delivery of a noncontingent electrical stimulus. Failure to respond to the noncontingent stimulus within 7.5 s resulted in the onset of the intertrial interval. Responding during the intertrial interval delayed the onset of the next trial by 12.5 s. Current levels were varied in alternating descending and ascending series. A set of three trials was presented for each current intensity. Current intensities were altered in 5-μA steps. In each testing session, four alternating descending and ascending series were presented. The threshold for each series was defined as the midpoint between two consecutive current intensities that yielded “positive scores” (animals responded for at least two of the three trials) and two consecutive current intensities that yielded “negative scores” (animals did not respond for two or more of the three trials). The overall threshold of the session was defined as the mean of the thresholds for the four individual series. Each testing session was ~30 min in duration. The time between the onset of the noncontingent stimulus and a positive response was recorded as the response latency. The response latency for each test session was defined as the mean response latency of all trials during which a positive response occurred. After establishment of stable ICSS reward thresholds, rats were tested in the ICSS procedure once daily except for the spontaneous nicotine withdrawal experiment when rats were tested at time points according to the experimental design.

Intracerebral Injection Procedure. All injections were administered bilaterally in a volume of 0.5 μl/side given over 66 s through 17-mm injectors. The injectors were connected to calibrated pressure ejection systems preloaded with drug solution and protruded 3 mm below the ends of the cannulas into the VTA. After infusion, the injectors were kept in place for an additional 60 s to allow for drug diffusion and to minimize diffusion along the injection tract when pulling out the injector. Injectors were then removed and replaced with 14-mm wire stylets, and the animals were placed directly into the ICSS testing apparatus. Injections were made using a microinfusion pump (model 975; Harvard Apparatus Inc., Holliston, MA).

Systemic Drug Administration Experiments. These experiments investigated whether nicotine withdrawal, as measured by elevations in ICSS thresholds, could be precipitated in nicotine-naive rats and their corresponding nicotine-naive control group were then injected intraperitoneally with the mGlull receptor agonist LY314582 (0, 2.5, 0.5, and 7.5 mg/kg; n = 9 nicotine, n = 11 control), the mGlur5 receptor antagonist MPEP (0, 0.01, 0.05, and 0.1 mg/kg; n = 8 nicotine, n = 7 vehicle or 0, 0.5, 1, 2, and 3 mg/kg; n = 13 nicotine, n = 13 vehicle), the NMDA receptor antagonist dizocilpine (0.01, 0.05, 0.1, 0.175, and 0.2 mg/kg; n = 10 nicotine, n = 9 control), or the AMPA/kainate receptor antagonist NBQX (0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.5, and 1 mg/kg; n = 10 nicotine, n = 12 control) according to within-subjects Latin square designs and ICSS thresholds were evaluated 30 min later. A minimum of 48 h was allowed between each injection in the Latin square design, during which ICSS thresholds continued to be measured daily, to ensure that ICSS thresholds returned to baseline levels before the next drug administration. The doses of LY314582 and MPEP were chosen based on a previous study demonstrating that ≥10 mg/kg LY314582 and ≥3 mg/kg MPEP elevated ICSS thresholds in drug-naive rats (Harrison et al., 2002). For the potential demonstration of statistical interaction effects, it was important to include doses of the test drugs that did not alter thresholds under baseline conditions.

Intraventral Tegmental Area Administration Experiment. After stable baseline ICSS responding was achieved (≥10% variation in thresholds for three consecutive days), rats (n = 15) with bilateral cannulas directed toward the VTA were allocated to two groups such that there were no differences in mean baseline reward thresholds or body weight between groups. One group was then prepared with subcutaneous osmotic mini-pumps delivering vehicle and a second group with mini-pumps delivering nicotine (3.16 mg/kg/day nicotine-free base). Animals again were tested in the ICSS paradigm each day for 7 days before drug treatment. Both groups of rats were then injected directly into the VTA, as described above, with LY314582 (0, 10, 50, and 100 ng/side; n = 9 nicotine, n = 8 control), or the AMPA/kainate receptor antagonist NBQX (0, 0.01, 0.025, 0.05, 0.075, 0.1, 0.5, and 1 mg/kg; n = 10 nicotine, n = 12 control) according to a within-subjects Latin square design, and ICSS reward thresholds were evaluated immediately post injection. There was a minimum 48-h interval between each injection, during which ICSS thresholds continued to be measured, to allow thresholds to return to baseline levels before further drug tests. At the conclusion of the experiment, all animals were anesthetized and their brains removed and immediately placed on ice. The brains were cut in 50-μm sections, and placements of the injectors and the electrodes were examined (Fig. 1 for histological verification of injection sites). Only those rats with injection tips located within the VTA were included in statistical analyses.

Spontaneous Nicotine Withdrawal Experiment. Osmotic mini-pumps were surgically removed from nicotine-treated rats (n = 15) (defined as rats having been prepared with mini-pumps delivering 3.16 mg/kg/day nicotine free-base for at least 7 days) or corresponding control rats (n = 17; rats prepared with vehicle-containing mini-pumps). All rats were then tested in the ICSS procedure at 12, 18, 24, 36, 48, and 72 h after the removal of osmotic mini-pumps. These time points were chosen based on the time course of threshold elevations previously observed during spontaneous nicotine withdrawal after removal of nicotine-delivering osmotic mini-pumps (Harrison et al., 2001). Based on the ICSS reward thresholds obtained at the 12-h time point, nicotine-withdrawing rats were allocated to two groups such that there was no difference in the magnitude of reward threshold elevations between each group (117.67 ± 3.1%, n = 8; 119.93 ± 3.5%, n = 7). Similarly, control rats were allocated to two groups such that there was no difference in mean reward thresholds between these groups (106.45 ± 5.2%, n = 7; 103.63 ± 3.6%, n = 10). Thirty min before being tested at the 18-h
time point, one group of nicotine withdrawing and one group of control rats were injected with LY341495 (1 mg/kg); the remaining rats were injected with vehicle.

**Statistical Analyses.** Mean raw thresholds and response latencies (± S.E.M.) are presented for each experiment in the results section. For all experiments, except the spontaneous nicotine withdrawal experiment, percentage of change from baseline reward threshold was calculated by expressing the drug-influenced raw threshold scores as a percentage of the previous day’s threshold (i.e., a drug-free baseline threshold). These percentages of baseline scores were subjected to two-factor repeated-measures analyses of variance (ANOVA), with treatment drug dose as the within-subjects factor and pump content (nicotine or control) as the between-subjects factor. For the spontaneous nicotine withdrawal experiment, percentage change from baseline reward threshold was calculated by expressing the threshold scores obtained at each time point during withdrawal as a percentage of thresholds for each rat on the day immediately before mini-pump removal. These percentages of baseline scores were subjected to three-factor repeated measures ANOVA. The within-subjects factor was the time after mini-pump removal, and the two between-subjects factors were pump content (nicotine or vehicle) and acute drug treatment (LY314582 or vehicle). For all experiments, response latency data were analyzed in the same manner as the threshold data. After statistically significant effects in the ANOVAs, post hoc comparisons among means were conducted with the Fisher’s least significant difference test. The level of significance was set at 0.05.

**Results**

**Systemic Administration of the mGluII Receptor Agonist LY314582 Precipitated Elevations in ICSS Thresholds in Nicotine-Treated but Not Control Rats.** Mean (±S.E.M.) raw reward thresholds before treatment with the mGluII receptor agonist LY314582 for control and nicotine-treated rats were 115.3 ± 12.2 and 113.5 ± 19.0 μA, respectively. Mean (±S.E.M.) raw response latencies for control and nicotine-treated rats were 3.38 ± 0.2 and 3.21 ± 0.14 μA, respectively. Intraperitoneal administration of LY314582 (2.5–7.5 mg/kg) elevated ICSS reward thresholds in nicotine-treated but not control rats. This effect was reflected in a statistically significant effect of group [F(1,18) = 7.43, p < 0.05], a significant effect of dose [F(3,54) = 5.02, p < 0.005], and a significant group × dose interaction [F(3,54) = 2.79, p < 0.05]. Post hoc analysis revealed that the highest dose of LY314582 (7.5 mg/kg) elevated reward thresholds in nicotine-treated rats compared with vehicle treatment (p < 0.01) and compared with control rats tested with the same dose (p < 0.01) (Fig. 2). In contrast to its effects on reward thresholds, LY314582 had no effect on response latencies in nicotine-treated or control rats [F(3,54) = 0.59, N.S.] at any dose tested (data not shown).

**Ventral Tegmental Area Administration of the mGluII Receptor Antagonist LY314582 Attenuated the Elevations in ICSS Thresholds Associated with Spontaneous Nicotine Withdrawal.** Mean (±S.E.M.) raw reward thresholds prior to mini-pump re-
moval for control and nicotine-treated rats were 105.9 ± 7.8 and 105.9 ± 11.0 μA, respectively. Mean (±S.E.M.) raw response latencies for control and nicotine-treated rats were 3.38 ± 0.1 and 3.36 ± 0.13 μA, respectively. Withdrawal from chronic nicotine treatment produced robust ICSS threshold elevations compared with control rats \( F_{1,27} = 15.3, p < 0.001 \) (Fig. 4A). Analysis of the significant group × dose × time interaction \( F_{15,135} = 3.3, p < 0.02 \) revealed the following. Nicotine-treated rats injected with vehicle demonstrated robust reward threshold elevations that reached a peak 24 h after mini-pump removal (Fig. 4A). However, administration of LY341495 30 min before the 18-h time point significantly attenuated the elevations in reward thresholds in nicotine-withdrawing rats \( p < 0.001 \) (Fig. 4A), without affecting thresholds in control rats (Fig. 4B). LY341495 had no effect on response latencies at any time point after injection \( F_{1,27} = 0.43, \text{N.S.} \) in nicotine-treated or control rats (data not shown).

The NMDA Receptor Antagonist Dizocilpine Lowered ICSS Thresholds Similarly in Nicotine-Treated and Control Rats. Mean (±S.E.M.) raw reward thresholds before treatment with the NMDA receptor antagonist dizocilpine for control and nicotine-treated rats were 88.9 ± 9.1 and 86.9 ± 3.2 μA, respectively. Mean (±S.E.M.) raw response latencies for control and nicotine-treated rats were 3.32 ± 0.06 and 3.10 ± 0.06 μA, respectively. As can be seen in Fig. 5A, dizocilpine (MK-801; 0.01–0.2 mg/kg) lowered ICSS reward thresholds in nicotine-treated and control rats \( F_{6,66} = 7.5, p < 0.0001 \), and there was no group × dose interaction \( F_{6,66} = 1.2, \text{N.S.} \). Doses of dizocilpine ≥0.2 mg/kg caused disruption in performance in the ICSS paradigm in both groups such that rats no longer responded for self-stimulation, and therefore doses higher than 0.2 mg/kg were not tested. Furthermore, dizocilpine did not precipitate withdrawal-like elevations in reward thresholds in nicotine-treated rats at any dose tested. Dizocilpine significantly increased response latencies \( F_{0.72} = 2.9, p < 0.05 \). Post hoc analysis demonstrated that as the dose of dizocilpine increased, so too did response latency, particularly in control rats, suggesting that performance was increasingly impaired at higher doses of dizocilpine (Fig. 5B).

The mGlu5 Receptor Antagonist MPEP Elevated ICSS Thresholds Similarly in Nicotine-Treated and Control Rats. Mean (±S.E.M.) raw reward thresholds before treatment with low doses of the mGlu5 receptor antagonist MPEP for control and nicotine-treated rats were 118.9 ± 9.3 and 98.4 ± 8.9 μA, respectively. Mean (±S.E.M.) raw response latencies for the low-dose MPEP experiment for control and nicotine-treated rats were 3.34 ± 0.09 and 3.43 ±
0.12 s, respectively. Mean (±S.E.M.) raw reward thresholds before treatment with high doses of MPEP for control and nicotine-treated rats were 112.9 ± 8.9 and 109.5 ± 8.5 µA, respectively. Mean (±S.E.M.) raw response latencies for the high-dose MPEP experiment for control and nicotine-treated rats were 3.27 ± 0.19 and 3.14 ± 0.07 s, respectively. Low doses of MPEP (0.01–0.1 mg/kg) did not affect ICSS reward thresholds \( F_{(3,39)} = 2.3, \text{N.S.} \) or response latencies \( F_{(3,39)} = 0.4, \text{N.S.} \) in nicotine-treated or control rats (data not shown). Higher doses of MPEP (0.5–3 mg/kg) elevated ICSS thresholds in nicotine-treated and control rats \( F_{(4,96)} = 8.4, p < 0.0001 \) (Fig. 6). However, MPEP elevated ICSS thresholds in both groups of rats by a similar magnitude (Fig. 6), and there was no group x dose interaction \( F_{(7,140)} = 0.7, \text{N.S.} \). MPEP (0.5–3 mg/kg) had no effect on response latencies \( F_{(4,96)} = 1.4, \text{N.S.} \) in either group (data not shown).

**The AMPA/Kainate Receptor Antagonist NBQX Precipitated Elevations in ICSS Thresholds in Nicotine-Treated but Not Control Rats.** Mean (±S.E.M.) raw reward thresholds prior to treatment with the AMPA/kainate receptor antagonist for control and nicotine-treated rats were 98.9 ± 10.0 and 98.5 ± 11.8 µA, respectively. Mean (±S.E.M.) raw response latencies for control and nicotine-treated rats were 3.21 ± 0.09 and 3.36 ± 0.15 µA, respectively. NBQX (0.01–1 mg/kg) significantly altered ICSS thresholds in nicotine-treated but not control rats (Fig. 7). This effect was reflected in a statistically significant effect of group \( F_{(1,20)} = 10.82, p < 0.005 \), a significant effect of dose \( F_{(7,140)} = 2.8, p < 0.01 \), and a significant group x dose interaction \( F_{(7,140)} = 2.11, p < 0.05 \). Post hoc analysis revealed a bimodal action of NBQX on ICSS thresholds in nicotine-treated rats. Low doses of NBQX (0.025–0.1 mg/kg) elevated thresholds in nicotine-treated rats, whereas higher doses of NBQX (0.5–1 mg/kg) were less effective and did not significantly elevate thresholds compared with vehicle treatment (Fig. 7). NBQX had no effect on response latencies in nicotine-treated or control rats at any dose tested \( F_{(7,140)} = 0.31, \text{N.S.} \) (data not shown).

**Fig. 5.** Effects of dizocilpine (MK-801) on ICSS thresholds and response latencies in nicotine-treated and control rats. A, data are expressed as mean (±S.E.M.) percentage change from baseline thresholds. B, data are expressed as mean (±S.E.M.) percentage change from baseline response latencies. *, \( p < 0.05 \); **, \( p < 0.01 \), different from corresponding vehicle- or nicotine-treated rats after vehicle injection.

**Fig. 6.** Effects of MPEP on ICSS thresholds in nicotine-treated and control rats. Data are expressed as mean (±S.E.M.) percentage change from baseline thresholds. ***, \( p < 0.01 \), different from corresponding vehicle- or nicotine-treated rats after vehicle injection.

**Fig. 7.** Effects of NBQX on ICSS thresholds in nicotine-treated and control rats. Data are expressed as mean (±S.E.M.) percentage change from baseline thresholds. *, \( p < 0.05 \); **, \( p < 0.01 \), different from corresponding vehicle- or nicotine-treated rats after vehicle injection. #, \( p < 0.05 \); ##, \( p < 0.01 \), different from control rats after injection with same dose of NBQX.
Discussion

Nicotine withdrawal precipitates an aversive abstinence syndrome in human smokers hypothesized to provide an important source of motivation contributing to the persistence of the smoking habit and relapse during abstinence (Kenny and Markou, 2001). The present data strongly suggest a role for group II metabotropic glutamate receptors in generating the reward deficits associated with nicotine withdrawal by demonstrating that activation of mGluII receptors precipitated ICSS threshold elevations in nicotine-dependent rats similar to those observed during spontaneous nicotine withdrawal. Furthermore, activation of mGluII receptors in the VTA also elevated thresholds in nicotine-dependent rats, providing further support for an important role of the VTA in mediating the actions of nicotine on reward pathways. Consistent with the above-mentioned information, blockade of mGluII receptors attenuated the reward deficits in rats undergoing spontaneous nicotine withdrawal. Previously, the mGluII receptor agonist LY354740 was shown to attenuate the increased auditory startle observed during spontaneous nicotine withdrawal (Helton et al., 1997). One possible explanation for these observations is that mGluII receptors located in different brain sites may differentially regulate various aspects of nicotine withdrawal.

Previously, the mGluII receptor agonist LY314582, which we found here to elevate reward thresholds in nicotine-dependent rats at doses ≤7.5 mg/kg, was shown to elevate ICSS thresholds in control rats at doses ≥10 mg/kg (Harrison et al., 2002). Therefore, the present observation that “subthreshold” doses of LY314582 precipitated withdrawal-like threshold elevations in nicotine-dependent but not control rats suggests that negative regulation of brain reward function by mGluII receptors was increased by prolonged nicotine treatment. One mechanism through which nicotine elicits its reinforcing effects is by increasing glutamatergic transmission in the VTA, thereby potentiating mesoaccumbens dopaminergic transmission (Schilstrom et al., 1998). Because mGluII receptors located in the VTA are presynaptic autoreceptors that decrease glutamate transmission (Bonci et al., 1997; Wigmore and Lacey, 1998), it is likely that the increased mGluII receptor sensitivity in the VTA observed in nicotine-treated rats occurred in response to prolonged activation of excitatory glutamate transmission by nicotine in this brain site, perhaps to counter this effect. Thus, during nicotine withdrawal when the stimulatory effects of nicotine on excitatory glutamate transmission were no longer present, increased mGluII receptor function would be expected to decrease glutamate transmission and thereby decrease the activity of this brain reward substrate. Recent electrophysiological studies are consistent with this hypothesis. For instance, chronic opiate treatment increased the inhibitory effects of mGluII receptor agonists on excitatory glutamate currents in VTA dopamine neurons (Manzoni and Williams, 1999), and in nucleus accumbens neurons (Martin et al., 1999). Nevertheless, it is possible mGluII receptors are also located on non glutamatergic terminals (e.g., serotonergic and cholinergic neurons) and that activation of mGluII receptors precipitated nicotine withdrawal by decreasing the release of neurotransmitters other than glutamate. Indeed, nicotine withdrawal-induced threshold elevations were attenuated by coadministration of fluoxetine, a selective serotonin reuptake inhibitor, and 4-(2’-methoxyphenyl)-1-[2’(N’-2’-pyridinyl)-p-iodo-benzamido]ethylpiperazine (P-MPPI), a serotonin-1A receptor antagonist, suggesting that decreased serotonergic transmission also contributes to the reward deficits associated with nicotine withdrawal (Harrison et al., 2001).

To further investigate a potential role of decreased glutamatergic transmission in the reward deficits associated with nicotine withdrawal, we examined whether antagonists at postsynaptic glutamate receptors precipitated withdrawal-like threshold elevations in nicotine-dependent rats similar to activation of mGluII receptors. At low doses the AMPA/kainate receptor antagonist NBQX precipitated threshold elevations in nicotine-treated but not controls rats. Under “normal” baseline conditions, AMPA receptors are the primary regulators of excitatory glutamate transmission throughout the mesoaccumbens reward pathway (Pennartz et al., 1990). Furthermore, AMPA receptor overexpression in the VTA increased, whereas AMPA receptor blockade decreased, the rewarding actions of drugs of abuse (Carlezon et al., 1997; Xi and Stein, 2002). These observations suggest that AMPA receptors positively modulate brain reward function. Conversely, AMPA receptor antagonists elicit an intrinsic rewarding action after VTA administration (David et al., 1998), suggesting that AMPA receptors may also negatively regulate brain reward function under baseline conditions. Indeed, AMPA receptors are located on dopamine and GABAergic neurons in the VTA (Wang and French, 1993, 1995), where they modulate mesoaccumbens dopamine transmission in an opposite manner. Therefore, it is possible that NBQX had no effects in control rats because it simultaneously blocked populations of AMPA/kainate receptors that positively and negatively regulate reward function. However, the sensitivity of nicotine-treated rats to NBQX suggests a scenario in which the development of nicotine dependence led to compensatory decreases in the number and/or function of those AMPA/kainate receptors that positively regulate brain reward function, perhaps to counter the prolonged stimulatory effects of nicotine on reward pathways. Consistent with this hypothesis, prolonged nicotine exposure decreased AMPA receptor immunoreactivity in the VTA and nucleus accumbens (Lee et al., 2002). Alternatively, it is possible that a “silent” population of AMPA/kainate receptors was recruited during prolonged nicotine exposure (Isaac et al., 1995), resulting in increased regulation of reward circuits by AMPA/kainate receptors. Regardless of the mechanism, these data suggest that decreased glutamatergic transmission at AMPA/kainate receptors contributes to the threshold elevations observed in nicotine withdrawing rats.

There is considerable evidence that NMDA receptors play an important role in mediating the stimulatory effects of nicotine on mesoaccumbens dopamine transmission (Grillner and Svensson, 2000). Therefore, it might have been expected that prolonged nicotine treatment may have resulted in adaptations in the function/number of NMDA receptors such that their blockade precipitated withdrawal-like threshold elevations in nicotine-dependent rats but not controls similar to AMPA/Kainate receptor blockade. Nevertheless, this did not seem to be the case. Similar to previous reports (Carlezon and Wise, 1993), NMDA receptor blockade lowered thresholds in nicotine-dependent and control rats, indicating a rewarding action. At no dose tested did the NMDA receptor antagonist dizocilpine elevate thresholds in either nicotine-
treated or control rats. Interestingly, dextroamphetamine tended to lower thresholds by a greater magnitude in nicotine-treated rats, suggesting they were slightly more sensitive to dextroamphetamine’s reward-facilitating effects. Furthermore, higher doses of dextroamphetamine elevated response latencies in control but not nicotine-dependent control rats, suggesting that prolonged nicotine treatment attenuated the performance-disrupting effects of dextroamphetamine. Nevertheless, based on the present data it is unlikely that decreased glutamatergic transmission at NMDA receptors contributes to the threshold elevations associated with nicotine withdrawal.

Recently, mGlur5 receptors, which are primarily located postsynaptically throughout the mesocorticolimbic system (Wigmore and Lacey, 1998), were shown to block the reinforcing effects of drugs of abuse, including nicotine (Chiamulera et al., 2001; Paterson et al., 2003). Therefore, we also investigated the role of mGlur5 receptors in nicotine withdrawal. At low doses, the mGlur5 receptor antagonist MPEP had no effect on ICSS thresholds, whereas higher doses elevated thresholds in nicotine-dependent and control rats (consistent with Harrison et al., 2002). Interestingly, MPEP tended to elevate thresholds by a greater magnitude in nicotine-dependent rats compared with control. However, because no dose of MPEP differentially elevated thresholds in nicotine-treated rats without also elevating thresholds similarly in control rats, these data indicate that mGlur5 receptors regulate baseline brain reward function in control and nicotine-treated rats, but are probably not involved in the threshold elevations associated with nicotine withdrawal.

Perhaps the most parsimonious explanation of the present observations is that prolonged, continuous nicotine exposure increased mGlurII receptor function, and decreased AMPA/kainate-mediated glutamate transmission in reward circuits, which contributed to the reward deficits observed during nicotine withdrawal. In contrast, recent investigations demonstrated that repeated, intermittent exposure to psychostimulants decreased mGlurII function, and increased AMPA/kainate receptor transmission in reward circuits (Giorgetti et al., 2001; Xi et al., 2002). Thus, it is possible that chronic nicotine and psychostimulant administration induce different alterations in glutamatergic transmission. Alternatively, this apparent discrepancy may be explained by the fact that the long-term behavioral effects of drugs of abuse are related to the dose-sensitive regimen (i.e., continuous or intermittent). Specifically, repeated intermittent exposure to addictive drugs can result in a progressive augmentation or “sensitization” in their behavioral effects (Pierce and Kalivas, 1997; Wolf, 1998). Conversely, more continuous exposure similar to that used in the present study, and similar to the pattern of prolonged nicotine exposure observed in smokers, engages counteradaptive “opponent processes” that decrease the acute behavioral effects of addictive drugs (i.e., “tolerance”), and leads to the expression of an aversive withdrawal syndrome upon cessation (Koob and Le Moal, 2001). It has been proposed that sensitization may be important in the early stages of drug addiction, when intake is intermittent, whereas tolerance and withdrawal may be more important in later stages of drug dependence, as drug intake progressively increases (Koob and Le Moal, 2001; Kenny et al., 2003).

Based on the above-mentioned information, it is an interesting possibility that an initial increase, followed by a prolonged decrease in glutamatergic transmission, mediated by mGlurII and AMPA/kainate receptors, may be involved in the initiation and maintenance of the drug-taking habit, respectively. Thus, it will be of interest to investigate whether other major drugs of abuse also increase the regulation of brain reward function by mGlurII receptors.

In conclusion, the present data suggest that mGlurII receptors play an important role in generating the reward deficits associated with nicotine withdrawal. Furthermore, it is likely that mGlurII receptors generated these deficits, at least in part, by decreasing glutamate transmission at AMPA/kainate receptors. Thus, because the reward deficits associated with drug withdrawal are thought to play such a crucial role in drug addiction (Ahmed et al., 2002; Kenny et al., 2003), these data suggest that mGlurII and AMPA/kainate glutamate receptors may prove to be useful therapeutic targets for the treatment of nicotine addiction.

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