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ScienceDirect
Pharmacology, Biochemistry and Behavior 88 (2007) 82–88
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Continuous intracerebroventricular infusion of the competitive NMDA receptor antagonist, LY235959, facilitates escalation of cocaine self-administration and increases break point for cocaine in Sprague–Dawley rats☆

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Received 28 March 2007; received in revised form 5 July 2007; accepted 11 July 2007
Available online 21 July 2007

Abstract

Although escalation of consumption is an important characteristic of cocaine dependence, the neurobiological mechanisms that mediate this phenomenon have not been fully described. In this study, we used male, Sprague–Dawley rats to measure the effects of acute and continuous intracerebroventricular (ICV) administration of the competitive NMDA receptor antagonist, LY235959, on cocaine self-administration behavior under various schedules of reinforcement and access conditions. Single ICV infusions of LY235959 (0.03–0.3 μg/5 μl) produced dose-dependent and statistically significant decreases in the number of cocaine infusions earned under a progressive ratio schedule of reinforcement. In a second experiment, vehicle or LY235959 (0.2–0.3 μg/day) was continuously administered ICV to rats via surgically-implanted subcutaneous osmotic minipump/intracranial cannula assemblies. Both vehicle- and LY235959-treated rats significantly escalated cocaine self-administration over the 10 long access sessions; however, rats treated with LY235959 escalated cocaine self-administration faster and to a greater degree than vehicle-treated rats. There was a statistically significant increase in cocaine infusions earned under the PR schedule in LY235959-treated rats, but not vehicle-treated rats, after 10 long access cocaine self-administration sessions. These data support the hypothesis that escalation of cocaine consumption is mediated by hypo-glutamatergic tone in the central nervous system and this facilitation of escalation is associated with an increase in motivation to respond for cocaine.

Keywords: Escalation; Tolerance; Sensitization; Glutamate; Osmotic minipump; Lateral ventricle; Stimulant; Progressive ratio

1. Introduction

An increase in the consumption of cocaine over time is one feature of human cocaine dependence (i.e., addiction). Preclinical models that allow rats to self-administer cocaine in extended access sessions are being used to identify underlying neurobiological and behavioral mechanisms that contribute to escalation of cocaine consumption (e.g., Ahmed et al., 2002; Ahmed and Koob, 2004; Mantsch et al., 2000; Mateo et al., 2005; Mutschler et al., 2001). In one such model, trained rats are subsequently permitted short (1 h) or long (6 h) access to cocaine in daily self-administration sessions (Ahmed and Koob, 1998). Under these conditions, only rats that self-administer cocaine during long access sessions increase consumption of cocaine. After escalation, rats self-administer more of a range of cocaine doses (shifting the dose-infusion curve "upward") and, under some conditions, attain higher break points under a progressive ratio (PR) schedule of reinforcement (Ahmed and Koob, 1998; Paterson and Markou, 2003; but see Liu et al., 2005). These findings suggest that escalation of consumption can be accompanied by an increase in the reinforcing effectiveness of cocaine under some conditions.

☆ This work was supported by United States Public Health Service Grant DA14389 (RMA) from the National Institute on Drug Abuse, a UCDHSC Psychology Faculty Fund for Undergraduate Research award (EMA), and a UCDHSC Undergraduate Research Opportunity Program award (EMA). A Preliminary report of these data was presented at the 2006 annual meeting of the Society for Neuroscience (Atlanta, GA).

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doi:10.1016/j.pbb.2007.07.009

Studies in our laboratory show that *continuous* subcutaneous infusion of the competitive (NMDA) receptor antagonist, LY235959, *facilitates* escalation of cocaine consumption when rats self-administer cocaine during long access (i.e., 6 h) sessions (Allen et al., 2007). In that study, both long access cocaine self-administration and continuous LY235959 administration led to apparent "upward" shifts in cocaine dose–consumption curves (representing mg/kg cocaine intake at each self-administered dose of cocaine) when these data were collected following the escalation phase of the experiment. These dose–consumption curves were collected to assess changes in the reinforcing effectiveness of cocaine. However, some authors have argued it is difficult to use data like these, generated with a continuous reinforcement schedule, to interpret changes in the reinforcing effectiveness of a drug (e.g., Katz and Higgins, 2004). Thus, additional data were needed to confirm or refute the hypothesis that a decrease in glutamate function may underlie *both* escalation of cocaine consumption *and* changes in the reinforcing effectiveness of cocaine. Break point in rats that respond for cocaine under a PR schedule of reinforcement provides another, perhaps more direct measure of the reinforcing effectiveness of cocaine (Richardson and Roberts, 1996). Increases in break point for cocaine following various extended access histories of cocaine self-administration have been measured and support the hypothesis that cocaine escalation is accompanied by an increase in the reinforcing effectiveness of cocaine under some conditions (Paterson and Markou, 2003; but see Liu et al., 2005).

In the present study, rats' motivation to self-administer cocaine was measured with a PR schedule of reinforcement, both before and after 10 long access cocaine self-administration sessions during which rats received continuous intracerebroventricular (ICV) infusions of LY235959. The effect of acute ICV infusion of LY235959 on responding under the PR schedule was also measured in a separate group of rats. The present study had three aims. The first aim was to systematically replicate previous data that show continuous infusion of LY235959 increases the rate of cocaine escalation during 6 h sessions (Allen et al., 2007). The second aim was to extend these findings by measuring changes in the reinforcing effectiveness of cocaine using a PR schedule of reinforcement. Finally, this study assessed the feasibility of administering LY235959 via continuous intracranial infusion to rats that actively self-administer cocaine in order to establish a methodology for examining the specific neurobiological sites of action through which NMDA receptor antagonism facilitates escalation of cocaine consumption.

2. Materials and methods

2.1. Animals

The subjects in this study were male, Sprague–Dawley rats ($n=39$) previously trained to self-administer cocaine for an average of 16.3±0.5 sessions (mean±standard error of the mean, throughout). Of the 39 rats used in the study, 34 were purchased from Harlan (Indianapolis, IN) and weighed 275–324 g upon arrival at the animal colony. Five rats were born from Harlan timed pregnant females in the vivarium at the University of Colorado at Denver and Health Sciences Center downtown Denver campus, and were raised to the same weight range before the start of experimental manipulations. These five rats comprised 2 of the 8 rats in the acute ICV experiment and 3 of the 12 rats that received continuous ICV infusion of vehicle (see description of experiments below). A 12 h light/dark cycle was programmed in the vivarium, with lights on at 7:30 AM. Food and water were available *ad libitum* until the start of self-administration training. Thereafter, through training and the subsequent experiments, water was restricted to 30 ml/day and all rats were housed individually. All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

2.2. Catheter placement and cocaine self-administration training

Intravenous catheters were constructed in the laboratory and surgically implanted under ketamine (100 mg/kg, i.m.) and xylazine (10 mg/kg, i.m.) anesthesia using established procedures (Caine et al., 1993). Rats received acetaminophen in their drinking water (20 mg/ml) for 2 days as a post-surgical analgesic. Rats recovered from surgery for at least 1 week before self-administration training began. Catheters were flushed with 0.3 ml of bacteriostatic 0.9% sodium chloride solution that contained 16.7 USP Units/ml heparin, both before and after each self-administration session.

Rats self-administered cocaine during the light cycle in Plexiglas and metal operant conditioning chambers (29×24×21 cm; Med Associates, Incorporated; St. Albans, VT). Chambers were housed within sound-attenuating cabinets. Chambers contained two retractable levers mounted on the front wall with a fluid delivery trough located between the levers. Stimulus lights were positioned 6 cm above each lever. A tone presentation speaker (Sonalert Tone Generator, 2900 Hz), and a speaker for white noise (90 dB) were mounted 12 cm above the floor on the wall opposite to the levers. A houselight (100 mA) was mounted 6 cm above the tone speaker. Cocaine infusions were controlled by an electronic circuit that operated a computer controlled syringe pump. All behavioral events were monitored and controlled by a personal computer using MED-PC for Windows software (Med Associates, Inc.; St. Albans, VT).

Rats were trained in 2 h sessions during weekdays. Each self-administration training session began with the onset of a stimulus light located over a lever on the right side of the operant chamber and the extension of two retractable levers. Responses on the right side lever produced an intravenous infusion of cocaine (0.33 mg over 6 s) according to a fixed ratio 1 (FR1) schedule of reinforcement. The simultaneous onset of a tone (2900 Hz)-houselight (100 mA) conditioned stimulus complex (20 s) signaled the initiation of drug delivery. During the 20 s post reinforcement interval, responses on the right lever did not activate the pump. Responses on the left lever had no programmed consequences. During training, rats also responded for cocaine one to three times under a progressive ratio (PR) schedule of reinforcement. During these 5 h sessions, response

requirements under the PR schedule were 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, and 737 for cocaine infusions 1–25, respectively (Richardson and Roberts, 1996).

2.3. Acute intracranial infusions

After training, rats ($n=8$) were surgically implanted with a 22-gauge guide cannula (model C313G; Plastics One, Inc., Roanoke, VA) under ketamine (100 mg/kg)/xylazine (10 mg/kg) anesthesia. Cannulae were placed into the right lateral ventricle using the following coordinates relative to bregma: 1.5 mm posterior; 1.0 mm lateral; 3.2 mm below the horizontal plane of bregma. Guide cannulae were glued to three small screws embedded in the skull using a cyanoacrylate adhesive and accelerating solution (Loctite 382 and Loctite 712, respectively; Henkel Loctite Corp., Rocky Hill, CT) and then were embedded within dental cement (Jet Acrylic; Lang Dental Manufacturing Company, Inc., Wheeling, IL). After at least 72 h recovery, the effect of acute intracranial infusions of sterile saline vehicle or LY235959 (0.03–0.3 μg in 5 μl over 30 s) on responding under a PR schedule of cocaine reinforcement (0.33 mg/infusion) was measured. Infusion cannulae (which extended 0.5 mm below the distal end of the guide cannulae) were connected with microbore tubing to a 10 μl Hamilton syringe (model 1801RN; Hamilton Company, Reno NV).

Typically, rats were given ICV infusions and tested under the PR schedule two to three times per week: rats responded under the FR1 training schedule on all other days. Most rats were administered LY235959 in the following dose order: 0.1, 0.3, 0.03 μg. The effect of vehicle infusion on responding under the PR schedule was measured at least twice in each rat, including once before and once interspersed with LY235959 testing. Cannulae were left in place for 1 min after each 30 s infusion. Self-administration sessions began 5 min after each infusion. After ICV LY235959 tests, four of the eight rats used in this experiment were injected subcutaneously (sc) with saline vehicle or 6 mg/kg LY235959, in counterbalanced order, 30 min prior to the start of PR sessions in order to directly compare the effects of ICV and sc LY235959 in this study.

2.4. Continuous intracranial infusions

In a second experiment, rats self-administered cocaine during 10, 6 h self-administration sessions under an FR1 schedule of reinforcement, and during a post-escalation PR session, while receiving continuous ICV infusion of vehicle ($n=14$), 0.2 ($n=7$), or 0.3 μg/day LY235959 ($n=10$). Alzet osmotic minipumps (model 2002; Alza Corporation, Palo Alto, CA) connected via polyethelene tubing to intracranial cannulae (Alzet Brain Infusion Kits I or II) delivered vehicle or LY235959 to the right lateral ventricle at a continuous rate of 0.5 μl/h. The coordinates used were the same as those listed above, with the distal end of the implanted cannula ending 3.7 mm below the horizontal plane at bregma. Rats recovered for 72 h before the start of this experiment. Rats self-administered cocaine (0.33 mg/infusion) under the 6 h FR1 schedule during weekdays and under a PR schedule of reinforcement on Saturdays.

2.5. Exclusions and exceptions

None of the rats in the acute ICV experiments ($n=8$) were excluded from the final data analysis. Seven of the 31 rats in the escalation experiment were excluded from the final data analysis (vehicle pump, $n=2$; 0.2 μg/day LY235959 pump, $n=1$; 0.3 μg/day LY235959 pump, $n=4$). Three of these rats had a catheter fail during the experiment (vehicle pump, $n=2$; 0.2 μg/day LY235959 pump, $n=1$). Four rats treated with 0.3 μg/day LY235959 were euthanized before the end of the experiment. Two of these rats did not respond in self-administration sessions after pump implantation, and the other two experienced adverse events during stereotaxic surgery or post-surgical healing. All 24 rats self-administered cocaine under the PR schedule after 10, 6 h sessions. Eighteen of the 24 rats self-administered cocaine under the PR schedule after escalation session five, also. These data are not included in the final analysis. Finally, nine of the 31 rats that completed these experiments received intravenous infusions of morphine (10 mg/kg, $n=1$; vehicle pump group), intravenous infusions of cocaine (1 mg/kg, $n=3$; 0.2 μg/day LY235959 pump group), or subcutaneous injections of cocaine (20 mg/kg, $n=5$: acute ICV infusions group, $n=2$; vehicle pump group, $n=2$; 0.2 μg/day LY235959 pump group, $n=1$) within conditioned place preference apparatuses prior to the start of self-administration training, and thus were not drug naïve.

2.6. Verification of ICV cannula placements

Several days after recovering from surgery, rats implanted with single ICV guide cannulae ($n=8$; acute infusions study) were given an ICV infusion of Angiotensin II (20 μg/5 μl) and returned to their cages without access to their water bottles. Five min later, water bottles were returned and rats' drinking behavior was observed. All eight rats exhibited immediate and vigorous drinking behavior, verifying that the ICV infusions reached the ventricular system. Placement of guide cannulae in the continuous infusion study was verified post-mortem in a subset of rats. First, the tubing that connected the minipump to the infusion cannula was exposed in euthanized rats and was cut approximately 2 cm from its point of entry into the dental cement cap. Then, cresyl violet dye (0.15 ml) was injected through the tubing via a 1 ml syringe fitted to a 20 gauge blunt end tubing adapter. Finally, brains were extracted and bisected coronally to reveal the lateral ventricles. Cannula placement was verified by a clearly and selectively stained ventricular lining in each one of the last six rats to complete the experiment (vehicle infusion, $n=3$; 0.3 μg/day LY235959, $n=3$).

2.7. Data analysis

Data were analyzed using SPSS for Windows, version 14.0. Repeated measures analysis of variance (ANOVA) was used throughout. When the assumption of sphericity was violated for

a particular repeated-measures analysis, as revealed by Mauchly's test statistic, tests of significance were based on the more conservative Huynh–Feldt corrected degrees of freedom. The symbol, [a], indicates Huynh–Feldt corrected values throughout the text. When significant main and interaction effects were revealed by repeated-measures ANOVA, tests for between-groups significance were made using one-way ANOVA and independent-samples *t*-tests (significance set at $P<0.05$ in two-tailed *t*-tests). LY235959 dose was treated as a within-subjects independent variable for the acute infusions experiment and a between-subjects independent variable for the escalation experiment. For the escalation experiment, long access session and PR session were treated as within-subjects independent variables, and cocaine intake (mg/kg) and break point-associated infusions earned under the PR schedule of reinforcement were treated as the dependent measures in the statistical analyses. Break point was defined as the last response ratio completed before a period of one hour in which no infusions were earned.

Due to attrition within each LY235959 treatment group, and the similar effect produced by these two doses of LY235959, LY235959-treated rats (0.2 μg/day, $n=6$; 0.3 μg/day, $n=6$) were combined into one LY235959 treatment group ($n=12$) and their data compared with vehicle-infused rats ($n=12$) in the final data analysis. Three of the 24 rats in the escalation experiment (vehicle-infused, $n=2$; LY235959-infused, $n=1$) self-administered only 2 infusions before reaching break point during the post-escalation PR session. This was unusual behavior for rats in this experiment, and it added considerable variability to the statistical analysis of pre- and post-escalation PR session data. These rats were excluded from the PR data analysis.

2.8. Drugs

The National Institute on Drug Abuse generously supplied cocaine hydrochloride. Cocaine was dissolved in a 0.9% NaCl solution, filtered, and treated with heparin sodium (1.67 USP Units/ml). LY235959 {(−)-6-phosphonomethyl-deca-hydroisoquinoline-3-carboxylic acid)} was from stock generously provided by The Lilly Research Laboratories (Indianapolis, IN) prior to its commercial availability through Tocris Bioscience (Ellisville, MO). Angiotensin II was purchased from Sigma-Aldrich (St. Louis, MO). Both were dissolved in sterile saline (0.9% NaCl).

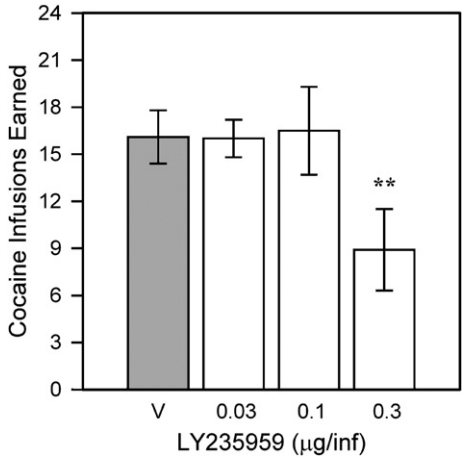

Fig. 1. Cocaine infusions earned (0.33 mg/infusion) under a progressive ratio schedule of reinforcement. $^{**}P<0.01$ vs. vehicle infusion. Rats received ICV infusions 5 min prior to the start of each session. Dose of LY235959 in μg/infusion is presented on the abscissa. V = vehicle.

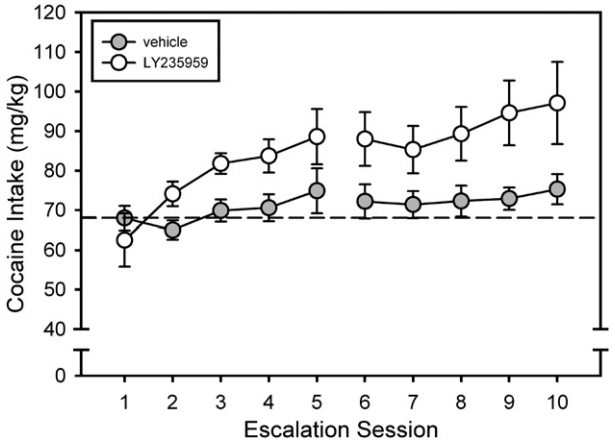

Fig. 2. Effect of continuous ICV infusion of vehicle ($n=12$) or LY235959 (0.2–0.3 μg/day; $n=12$) on escalation of cocaine intake. Rats self-administered cocaine (0.33 mg/infusion) under an FR1 schedule of reinforcement in 10, 6 h sessions on weekdays. The dashed line intersects mean cocaine intake for the vehicle-treated group during session one. Statistical analyses are described in the text.

3. Results

3.1. Effects of acute ICV infusion of LY235959 on cocaine self-administration under a PR schedule of reinforcement

Responding under the PR schedule of reinforcement was stable across the entire experiment. For example, mean infusions earned under the PR schedule was 16.4±1.1, 16.5±1.7, and 16.0±2.0 during the last PR session before cannula implantation, the first ICV vehicle infusion PR session, and the second ICV vehicle infusion PR session, respectively. As shown in Fig. 1, LY235959 dose-dependently and significantly decreased infusions earned under the PR schedule of reinforcement [$F(3,18)=8.122$, $P<0.01$]. Post-hoc testing revealed that relative to vehicle control, the 0.3 μg dose of LY235959 significantly decreased the number of cocaine infusions self-administered by rats ($P<0.01$). Four of the eight rats given ICV infusions subsequently were given sc injections of saline vehicle or 6 mg/kg LY235959, in counterbalanced order, and responding under the PR schedule of reinforcement was measured after each injection. As previously reported (Allen et al., 2005), 6 mg/kg LY235959 significantly decreased infusions earned under the PR schedule of reinforcement [15.5±2.1 vs. 6.0±1.9; $t(3)=9.922$, $P<0.01$].

3.2. Effects of continuous infusion of LY235959 on escalation of cocaine self-administration

Rats in the vehicle and LY235959 treatment groups were similar in many important ways at the start and end of these

experiments. For example, independent-samples *t*-tests revealed that there were no statistically significant between-group differences in body weight at the start of the experiment (prior to catheter implantation; 337.7±8.0 vs. 339.4±8.6, respectively), body weight at escalation session 10 (363.8±4.8 vs. 353.2±5.2, respectively), cocaine intake on the final baseline FR1 session (24.8±1.6 mg/kg vs. 24.5±1.2 mg/kg, respectively), the number of self-administration sessions completed before entering the escalation phase of the experiment (17.1±0.7 vs. 16.2±0.8, respectively), or in the number of cocaine infusions earned during the pre-escalation baseline PR session [17.8±1.4 (n=10) vs. 17.2±1.0 (n=11) respectively].

Fig. 2 shows cocaine intake (mg/kg) from the 10, 6 h cocaine self-administration sessions during which rats were given continuous ICV infusion of vehicle (n=12) or LY235959 (0.2–0.3 μg/day; n=12) through surgically-implanted osmotic minipump/intracranial cannulae assemblies. When data from the 10, 6 h self-administration sessions were analyzed, RMANOVA revealed a significant interaction between session and treatment [$^{a}F(4,82)=2.726$, $P<0.05$]. Both vehicle- and LY235959-infused rats increased escalation of cocaine consumption across the 10, 6 h sessions [$^{a}F(3,36)=3.51$, $P<0.05$ and $^{a}F(3,36)=6.703$, $P<0.01$, respectively]. However, vehicle-infused rats increased cocaine intake by approximately 16% over the ten sessions, from 61.8±3.0 mg/kg on session 1 to 71.8±3.5 mg/kg on session 10 ($P<0.01$), whereas LY235959-infused rats increased consumption of cocaine by nearly 66%, from 55.1±6.1 on session 1 to 91.2±10.0 on session 10 ($P<0.01$). Independent-samples *t*-tests revealed significant between-group differences at sessions 3 and 9 ($P<0.05$ for two-tailed test of significance) during which LY235959-treated rats self-administered more cocaine than vehicle-treated rats. There were also statistical trends for greater cocaine intake by LY235959-treated rats at sessions 4, 6, 8, and 10 ($P<0.1$ for two-tailed tests of significance).

3.3. Responding under a PR schedule of cocaine reinforcement before and after extended access to cocaine

Fig. 3 shows the number of infusions earned under the PR schedule at baseline (i.e., before long access cocaine self-administration sessions and continuous ICV infusion) and the number of infusions earned under the PR schedule after this exposure. When infusions earned were entered into an RMANOVA, a significant interaction between session and treatment condition was revealed [$F(1,19)=5.072$, $P<0.05$]. Post-hoc tests revealed no significant difference between vehicle- and LY235959-infused rats during the pre-escalation PR session (17.8±1.4 vs. 17.2±1.0, for vehicle- and LY235959-infused rats, respectively), but LY235959-infused rats self-administered more cocaine than vehicle-infused rats under the PR schedule post-escalation (17.5±1.0 vs. 21.3±1.2, for vehicle- and LY235959-infused rats, respectively; $P<0.05$). Further, when pre- and post-escalation PR session data were subjected to analysis with paired-samples *t*-tests, only LY235959-treated rats showed a significant change (i.e., an increase) in intake ($P<0.01$).

4. Discussion

Our published data show that acute subcutaneous injection of LY235959 dose-dependently *decreases* responding for cocaine under FR1 and PR schedules of reinforcement, suggesting it *decreases* the reinforcing effectiveness of cocaine (Allen et al., 2005). In contrast, continuous subcutaneous infusion of LY235959 increases *escalation* of cocaine consumption under long access self-administration conditions, as well as the self-administration of a range of cocaine doses, suggesting it leads to an *increase* in the reinforcing effectiveness of cocaine under these conditions (Allen et al., 2007). The present data set confirms and extends these findings to show that acute ICV infusion of LY235959 decreases responding for cocaine under a PR schedule whereas continuous ICV infusion of LY235959 both facilitates escalation and leads to an increase in responding for cocaine under the PR schedule of reinforcement.

LY235959 was up to 15,000 times more potent delivered ICV when compared with systemic administration under similar experimental conditions (Allen et al., 2005; Allen et al., 2007). Similarly, intrathecal infusions of LY235959 reduce phase 2 formalin-induced flinching behavior in rats at a dose approximately 20,000 times lower than the effective subcutaneous dose of LY235959 (0.001 nmol, i.t. vs. 20 nmol, s.c.; Davis and Inturrisi, 2001). Still, LY235959 produced the same behavioral effects after central and systemic administration, and it produces effects that are elicited by other NMDA receptor antagonists under other conditions (e.g., Allen and Dykstra, 2001; Bilsky et al., 1996; Schoepp et al., 1991). Thus, despite the potency differences, the effects produced by systemic and central administration of LY235959 can be attributed to its antagonist properties at the NMDA receptor and likely reflect the low bioavailability of the drug after systemic administration.

When we administered acute ICV infusions of LY235959 to rats, LY235959 dose-dependently decreased the number of infusions earned under the PR schedule of reinforcement. The

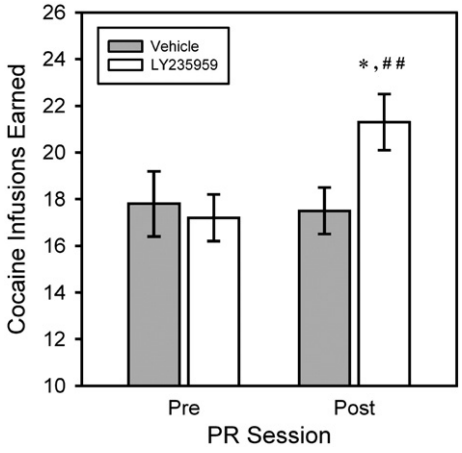

Fig. 3. Total cocaine infusions earned (0.33 mg/infusion) by rats under a progressive ratio schedule of reinforcement when responding was measured prior to (Pre) and 24 h after (Post) 10 long access sessions. *$P<0.05$, vehicle- vs. LY235959-infused rats, post-escalation. $^{\#\#}P<0.01$, pre- vs. post-escalation for LY235959-treated rats.

acute ICV administration of (+)-HA-966, a partial agonist for the strychnine-insensitive glycine site on the NMDA receptor, also decreased responding for cocaine under both FR and PR schedules of reinforcement (Cervo et al., 2004; Shoaib et al., 1995), though (+)-HA-966 also decreased responding for food under the PR schedule (Cervo et al., 2004). LY235959 has also been shown to decrease responding maintained by food in mice (Fischer and Dykstra, 2006). The effects of LY235959 on responding under FR and PR schedules of food reinforcement in rats are not known, but would be instructive.

One aim of the present study was to determine the feasibility of administering an NMDA receptor antagonist via continuous intracranial infusion to rats that self-administer cocaine under long access conditions, and in doing so, systematically replicate and extend our basic finding that LY235959 facilitates escalation of cocaine consumption. The experiments were not designed to measure the effect of continuous infusion of LY235959 in short-access control rats. We have previously shown that LY235959, when administered via continuous subcutaneous infusion through osmotic minipumps, increased cocaine consumption in short-access control rats, but consumption by these rats only differed significantly from short-access vehicle control rats when responding for a range of cocaine doses was measured after the escalation phase of the experiment (Allen et al., 2007). Because the effect of LY235959 in these rats was *emergent*, the effect of continuous infusion of LY235959 on cocaine self-administration may indicate a developmental process.

We do not have data that describe the mechanism (or mechanisms) for how acute infusion of LY235959 decreases consumption and the reinforcing effectiveness of cocaine whereas continuous infusion increases escalation and the reinforcing effectiveness of cocaine. It is possible that continuous NMDA receptor blockade produces compensatory neurobiological changes that result in effects opposite to those produced by acute administration. Such compensatory responses have been shown in a number of systems. For example, seven days continuous ICV infusion of the noncompetitive NMDA receptor antagonist, dizocilpine, increased NMDA receptor number fairly ubiquitously throughout the CNS (Oh et al., 2001; but see Scheggi et al., 2002). *In vitro*, chronic NMDA receptor blockade produces substantial axonal sprouting and increases the frequency of miniature excitatory postsynaptic currents in rat hippocampal slice cultures, and leads to an enhancement of pre-synaptic glutamate release in primary cultures of hippocampal neurons (McKinney et al., 1999; Bacci et al., 2001). More data are needed to address the precise mechanisms that account for the differences observed in rats that self-administer cocaine given acute and continuous exposure to NMDA receptor antagonists.

In the escalation experiment, cocaine intake by ICV vehicle-infused rats at escalation session 10 (71.8 mg/kg) was significantly greater than at escalation session one (61.8 mg/kg). Although a 16% change in intake might appear modest, we reliably observe statistically significant changes of about this magnitude after so few sessions, and we observe also that the degree of escalation can reach 30% within another four to five sessions (Allen et al., 2007; unpublished observations). In contrast, responding under the PR schedule did not change in these rats. It is possible, then, that our control rats did not complete enough long access cocaine self-administration sessions to reveal the increase in break point reported under some, but not all conditions (Paterson and Markou, 2003; but see Liu et al., 2005). Given these discrepant published results and the results from the present study, a thorough parametric analysis of the effects of duration of long access exposure on motivation to respond for cocaine, and of the correlation of changes in responding under a PR schedule with magnitude of escalation, would greatly advance our understanding of the role that long access exposure plays in modulating the reinforcing effectiveness of cocaine.

Although NMDA receptor antagonist treatment can interfere with acquisition of cocaine self-administration and the development of cocaine sensitization and conditioned place preferences (Cervo and Samanin, 1995; Karler et al., 1989; Schenk et al., 1993a,b) decreases in basal glutamate levels (Baker et al., 2003; Bell et al., 2000; Hotsenpiller et al., 2001; Moran et al., 2005), glutamate turnover rates (Smith et al., 2003), neural responses to iontophoretic application of glutamate (White et al., 1995), glutamate immunolabeling (Keys et al., 1998; Kozell and Meshul 2003), and the formation of long-term depression (LTD; Thomas et al., 2001) have all been measured following repeated cocaine exposure. How these data relate to escalation under long access cocaine self-administration conditions, during which exposure to cocaine is considerably greater, is not known.

In summary, taken together with our previously published results, the results of the present study suggest that continuous exposure to LY235959 during long access cocaine self-administration sessions leads to an increase in rate of escalation and in the reinforcing effectiveness of cocaine. Our success with the continuous intracranial infusion procedure prepares us for additional experiments in which LY235959 and other drugs can be administered continuously to specific corticomesolimbic structures in rats that self-administer cocaine in long access sessions.

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