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An examination of NMDA receptor contribution to conditioned responding evoked by the conditional stimulus effects of nicotine

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Abstract

Rationale—Research using a drug discriminated goal-tracking (DGT) task showed that the *N*-methyl-D-aspartate (NMDA) channel blocker MK-801 (dizocilpine) reduced the nicotine-evoked conditioned response (CR).

Objectives—Given the unknown mechanism of the effect, Experiment 1 replicated the MK-801 results and included tests with NMDA receptor ligands. Experiments 2a and 2b tested whether MK-801 pretreatment blocked DGT via a state-dependency effect.

Methods—In Experiment 1, adult male Sprague–Dawley rats received intermittent access to liquid sucrose following nicotine (0.4 mg base/kg); no sucrose was delivered on intermixed saline sessions. Conditioning was indicated by increased anticipatory dipper entries (goal-tracking) on nicotine compared to saline sessions. Antagonism and/or substitution tests were conducted with MK-801, phencyclidine, CGP 39551, d-CPPene (SDZ EAA 494), Ro 25,6981, L-701,324, ACPC, and NMDA. In Experiment 2a, rats received nicotine and sucrose on every session—no intermixed saline sessions without sucrose. Tests combined MK-801 or the non-competitive nicotinic acetylcholine receptor antagonist, mecamylamine with either nicotine or saline. Experiment 2b had sucrose delivered on saline sessions and no sucrose on intermixed nicotine sessions followed by MK-801 antagonism tests of the saline CS.

Results—MK-801 and phencyclidine dose-dependently attenuated the CR in Experiment 1. Ro-25,6981 enhanced the CR, but did not substitute for nicotine. Other ligands showed inconsistent effects. In Experiment 2a, MK-801 pretreatment reduced goal-tracking when given before nicotine and saline test sessions; mecamylamine pretreatment had no effect. In Experiment 2b, MK-801 dose-dependently attenuated the saline-evoked CR.

Conclusions—Combined, the results suggest that MK-801 blocks discriminated goal-tracking by virtue of state-changing properties.

Keywords

Appetitive Pavlovian conditioning; Discriminated goal-tracking; Mecamylamine; NMDA; NR2B; Drug discrimination; Smoking; State-dependent learning; Tobacco addiction

Introduction

Drug states and their interoceptive effects likely play an important role in addiction. As such, several animal models have been developed to study the interoceptive stimulus effects of drugs including nicotine. The most widely used model is the two-lever operant drug discrimination task with rats (see Rosecrans and Villanueva 1991; Smith and Stoleran 2009; Solinas et al. 2006) in which completion of a response requirement on one lever is typically reinforced in the drug state, whereas responding on the other lever is not reinforced. During intermixed vehicle sessions the reverse contingency applies. In rats, stimulus control by the internal state is evidenced by a majority of responding on the drug-appropriate lever (e.g., Chance et al. 1977; Desai et al. 2003; Stoleran et al. 1997; Wooters et al. 2009).

More recently, a discriminated goal-tracking (DGT) task with rats has been used to study the conditional stimulus (CS) effects of nicotine. In these studies, rats receive intermixed nicotine and saline sessions (e.g., Besheer et al. 2004; Bevins and Palmatier 2004). On nicotine sessions, access to sucrose is intermittently available in the chamber (see Murray et al. 2009b for water reinforcement). On saline sessions, no sucrose is available. The discrimination quickly develops as shown by increased anticipatory dipper entries (goal-tracking; e.g., Farwell and Ayres 1997) on nicotine compared to saline sessions. This conditioned response (CR) has been shown to be sensitive to manipulations such as nicotine training dose (Murray and Bevins 2007a, b), number and concentration (*w/v*) of sucrose delivered (Murray et al. 2009a; Wilkinson et al. 2006), and extinction training (Besheer et al. 2004; Murray and Bevins 2007b).

Of primary interest in the present report was the earlier finding that MK-801, a non-competitive channel blocker with high affinity for the *N*-methyl-D-aspartate (NMDA) glutamate receptor (Halliwell et al. 1989; Wong et al. 1986), dose-dependently reduced nicotine CS-evoked conditioned responding without affecting chamber activity (Murray and Bevins 2007a). The finding in the DGT task was particularly interesting given that pretreatment with MK-801 has not been found to block nicotine's control of responding in the two-lever operant drug discrimination task (Kim and Brioni 1995; Zakharova et al. 2005; see Zaniowska et al. (2008) for tests with agmatine). One possible explanation for this distinction is that the neurobiological mechanism underlying the CS effects of nicotine (DGT) differ somewhat from those mediating its discriminative stimulus effects in the two-lever task (cf. Murray et al. 2009b). If this hypothesis is correct, then more selective NMDA receptor antagonists should also block conditioned responding evoked by nicotine in the DGT task and provide additional information about the mechanism of the effect. As the NMDA receptor complex involves multiple subtypes and binding sites, we tested a variety of ligands with varying specificity to isolate the mechanism of this reduction in conditioned responding. Accordingly, in Experiment 1 we tested a slightly lower affinity NMDA channel blocker, phencyclidine (PCP; Wong et al. 1986), followed by competitive antagonists that bind to the NMDA but not the glycine/D-serine binding site on the NMDA receptor, CGP 39551 and d-CPPene (Fagg et al. 1990; Lowe et al. 1994), and a competitive antagonist specific to the presynaptic NR2B binding subtype, Ro 25-6981 (Fischer et al. 1997). We also tested L-701,324, an antagonist with specificity for the glycine/D-serine binding site of the NMDA receptor (Bristow et al. 1995), and 1-

aminocyclopropanecarboxylic acid hydrochloride (ACPC), a glycine/D-serine agonist with NMDA site antagonist actions (Sheinin et al. 2002), for antagonism and/or substitution for the CS effects of nicotine.

Another possibility for the difference seen between the DGT and two-lever tasks is that MK-801 has a state-dependency effect preventing recall of learning under some specific conditions (see Tzschentke and Schmidt 2000). Notably, nicotine does not appear to have this type of state-dependency. For example, rats previously trained to goal-track after pretreatment with nicotine (no intermixed saline sessions) did not display an overall reduction in responding when tested for the first time in the saline state. Similarly, rats trained to goal-track after pretreatment with saline (no intermixed nicotine sessions) did not show a significant reduction in responding when tested in the nicotine state. This outcome was taken to suggest that the nicotine-evoked CR in the DGT task was not due to state-dependent learning or recall (Bevins et al. 2007). If it had been, switching states would have profoundly disrupted responding. Experiment 2a expanded this observation to determine whether MK-801 would decrease a goal-tracking CR that was not under stimulus control of the nicotine state. Rats were first trained to goal-track following pretreatment with nicotine (no intermixed saline sessions) and then tested in a variety of 'different' states: saline, mecamylamine+ nicotine, mecamylamine+saline, MK-801+nicotine, MK-801+saline. The state-dependency account of a reduced CR predicts that MK-801 will reduce goal-tracking regardless of whether tested in combination with saline or nicotine. In contrast, saline in place of nicotine is not expected to reduce goal-tracking (cf. Bevins et al. 2007). Given that in this training regimen saline sessions are not intermixed, nicotine is not controlling the CR (chamber cues appear to be). As such, blocking nicotinic acetylcholine receptors with mecamylamine is not expected to reduce goal-tracking whether rats are given nicotine or saline on the test sessions. Finally, a reduction in goal-tracking brought about by MK-801 administration would suggest that MK-801 may be able to generally disrupt discriminated goal-tracking. To assess this hypothesis, a follow-up study using saline as the CS paired with sucrose deliveries and intermixed sucrose-free nicotine sessions (i.e., the opposite contingency as that in Experiment 1) was conducted to determine whether MK-801 pretreatment of the saline CS would reduce goal-tracking for sucrose similar to Experiment 1 and in Murray and Bevins (2007a).

Materials and methods

Subjects

Male Sprague–Dawley rats obtained from Harlan (Indianapolis, IN, USA) were housed individually in clear 48.3×26.7×20.3 cm (*l*×*w*×*h*) polycarbonate cages lined with aspen shavings. Water was continuously available in the home cage. Rats received food (Harlan Teklad Rodent Diet) after completion of daily sessions; the quantity was restricted as to maintain rats at 85% of free-feeding body weights. All sessions were conducted during the light portion of a 12 h light:dark cycle. Protocols were approved by the University of Nebraska—Lincoln Animal Care and Use Committee and followed the 'Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research' (National Research Council 2003).

Apparatus

Each of the eight conditioning chambers (ENV-008CT; Med Associates, Inc., St. Albans, VT, USA), measuring 30.5×24.1×21.0 cm (*l*×*w*×*h*), had aluminum sidewalls. The ceiling and front and back walls were clear polycarbonate. Each chamber was equipped with a 5.2×5.2×3.8 cm (*l*×*w*×*d*) recessed receptacle on one sidewall. A dipper arm raised a 0.1-ml cup of 26% sucrose solution (*w/v*) into the receptacle. An infrared emitter/detector unit, 1.2

cm into the receptacle and 3 cm from the chamber floor, monitored head entries. A second emitter/detector unit was mounted 14.5 cm from the sidewall with the receptacle and positioned 4 cm above the rod floor. Interruptions of this infrared beam provided an index of movement in the chamber. Each chamber was enclosed in a light- and sound-attenuating cubicle fitted with an exhaust fan. A personal computer with Med Associates interface and software (Med-PC for Windows, version IV) controlled sucrose deliveries and recorded dipper entries and activity.

Drugs

(–)-Nicotine hydrogen tartrate, mecamylamine hydrochloride, and 1-aminocyclopropanecarboxylic acid hydrochloride (ACPC) were purchased from Sigma (St. Louis, MO, USA). (+)-MK 801 maleate, Ro 25-6981 hydrochloride, CGP 39551, d-CPPene, *N*-methyl-D-aspartic acid (NMDA), and L-701,324 were purchased from Tocris Cookson, Inc. (Ellisville, MO, USA). PCP was generously provided by the NIDA drug supply program (RTI, Research Triangle Park, NC, USA). Vehicle, route of administration, injection volume, and injection-to-placement interval are described in Table 1. Nicotine doses are reported in base form; all other doses are reported in salt form.

Experiment 1

Discrimination training (Nic+/Sal–)—Sixteen experimentally naïve rats (363 ± 16 g before start of study) were handled for at least 3 min per day for 3 days. Rats were then given an injection of 0.4 mg/kg nicotine for the next 3 days in the home cage to attenuate the initial motor suppressant effects of nicotine (cf. Bevins et al. 2001). Daily training began the next day. Rats were administered nicotine or saline SC 5 min before placement in the chambers for 20 min. During nicotine sessions, there were 36 deliveries of 4-s access to sucrose. Four different programs were used to vary the time between sucrose deliveries. The average time before the first sucrose delivery was 137 s (range=124–152 s); the inter-sucrose interval thereafter ranged from 4 to 80 s (mean=25 s). In order to match timing on saline sessions, programs were matched to the nicotine programs such that 4-s ‘empty’ intervals occurred in place of sucrose deliveries. Session types and programs were intermixed with the restriction that no more than two nicotine or two saline sessions occurred in a row. Training continued for eight nicotine and eight saline sessions.

Antagonism and substitution testing—Following acquisition of the discrimination, rats entered testing. On the first four consecutive days of a 5-day cycle, rats received two nicotine and two saline training sessions as described earlier. If the discrimination criterion was met, then day 5 was a 4-min test session; sucrose was not available in testing. To qualify for testing, the dipper entry rate on each nicotine session had to be a minimum of 0.01 entries per second higher than each saline session across the four training days of a testing cycle (cf. Murray and Bevins 2007b). If the criterion was not met, then the rat remained in its home cage on day 5. The order of testing phases, drugs used, and doses is given in Table 2. Within each phase, test order of each dose was randomized for each rat. A rat completed one phase before progressing to the next phase.

Experiment 2a

Excitatory conditioning (no saline sessions)—Eight experimentally naïve rats (349 ± 21 g before start of study) were handled, maintained, and given pre-training nicotine as in Experiment 1. Daily training sessions began the day following the last nicotine injection. Rats were administered saline (IP) 25 min before 0.4 mg/kg nicotine (SC); rats were placed in the chamber 5 min after the nicotine injection. Saline injections were given to familiarize rats with IP administration and the timing of the injections in the testing phase.

During the 20-min sessions, there were 36 deliveries of 4-s access to sucrose. The programs that provided access to sucrose from Experiment 1 were used here. There were no saline (i.e., sucrose-free) sessions. Excitatory conditioning continued for 20 days.

State-change testing—Testing of different drug states followed conditioning. In order to qualify for a test session, conditioned responding had to be stable. Stable was defined as no statistically significant difference in rate of dipper entries for at least three training sessions. Rats were given two injections before each 4-min sucrose-free test. Drug details for the tests are provided in Table 3. Test order assignment was randomized for each rat. Training intervened between each test to insure that responding was stable.

Experiment 2b

Discrimination training (Sal+/Nic–)—Seven experimentally naïve rats (292 ± 2 g before start of study) were handled, maintained, and trained as described in Experiment 1 except for one major difference—sucrose was delivered on saline sessions, not on intermixed 0.4 mg/kg nicotine sessions (nicotine as a CS–; see Besheer et al. 2004). Training continued for eight nicotine and eight saline sessions.

Antagonism testing—Qualification for testing was established as described in Experiment 1. Each rat was tested on 0, 0.03, 0.1, and 0.2 mg/kg MK-801 administered before saline test sessions in a unique random order.

Dependent measures

The dependent measure for training was the rate of dipper entries per second before the first sucrose delivery or equivalent time on sucrose-free sessions. The dependent measure for test sessions was dipper entry rate in the first 2 min of the test; this interval approximates the average time before the first sucrose delivery during the training phase (cf. Besheer et al. 2004). Chamber activity, defined as rate of beam breaks per second in the time comparable to that for dipper entries, was also monitored.

Data analysis

For Experiments 1 and 2b, acquisition of the discrimination was confirmed using a paired *t* test to compare the mean (*M*) of the last three nicotine and saline training sessions. For generalization, antagonism, and substitution testing, one-way analyses of variance (ANOVAs) compared dose of ligand for dipper entry and activity rates. Significant ANOVAs were followed by Dunnett's multiple-comparison tests contrasting each test dose with its corresponding vehicle baseline. Significant Dunnett's tests were followed with paired *t* tests comparing the effective dose with the training baseline to determine if blockade was partial or full (effect reduced but not to the level of baseline responding, or reduced to the level of baseline responding, respectively). Median effective doses (ED_{50} s) were calculated on the linear portions of nicotine generalization curve and on antagonism tests that resulted in full blockade of conditioned responding. In Experiment 2a, stability of goal-tracking was assessed using one-way ANOVAs. For testing, one-way ANOVAs compared the drug states for dipper entry and activity rates. A significant ANOVA during testing was followed by Dunnett's multiple-comparison tests contrasting each test condition to the baseline conditioning state (i.e., Sal-Nic). For all experiments, statistical significance was declared at $p < 0.05$.

Results

Experiment 1

Discrimination training—Dipper entry rates were higher on nicotine than saline by the end of training, $t(15)=7.65$, $p<0.001$ ($M_s=0.178\pm0.02$ vs. 0.081 ± 0.01 on nicotine vs. saline sessions, respectively). There was no difference in activity, $t<1$ ($M_s=0.388\pm0.03$ and 0.381 ± 0.04 on nicotine and saline sessions, respectively).

Antagonism and substitution testing

Nicotine generalization: As shown in Fig. 1a, there was an increase in conditioned responding with 0.2 mg/kg, followed by a reduction in conditioned responding as the nicotine dose decreased toward saline, $F(5,75)=13.54$, $p<0.001$. Post-hoc analysis confirmed this impression of increased responding at 0.2 mg/kg nicotine. Additionally, saline and 0.025 mg/kg evoked lower responding than 0.4 mg/kg. The ED_{50} for nicotine was 0.084 mg/kg. There was an effect of nicotine on chamber activity, $F(5,75)=3.75$, $p<0.01$ (see Table 4). Activity on 0.1 and 0.2 mg/kg nicotine was higher than responding on saline; there were no differences in activity when compared with 0.4 mg/kg.

MK-801 and PCP antagonism: MK-801 and PCP dose-dependently decreased nicotine-evoked conditioned responding (Fig. 1b). For MK-801, there was a main effect of dose, $F(3,42)=14.26$, $p<0.001$, with subsequent contrasts indicating that 0.1 and 0.2 mg/kg MK-801 significantly reduced the CR relative to nicotine alone. Follow-up t tests compared 0.1 and 0.2 mg/kg doses of MK-801 to a saline baseline ($M=0.035\pm0.004$) generated using the last saline training session for each rat before testing with 2 mg/kg PCP. Antagonism with 0.1 mg/kg MK-801 was partial, $t(14)=3.37$, $p<0.01$, yet antagonism with 0.2 mg/kg was full, $t<1$. The ED_{50} for MK-801 was 0.104 mg/kg. For PCP, there was a main effect of dose, $F(3,42)=3.44$, $p<0.05$. The 2 mg/kg PCP dose reduced goal-tracking evoked by the nicotine CS, but that reduction was only partial, $t(14)=2.84$, $p<0.05$. PCP at 3 mg/kg profoundly inhibited locomotor activity (data not shown) in the first few rats tested so we ceased testing doses greater than 2 mg/kg. Table 4 shows neither MK-801 nor PCP affected chamber activity at the doses that were fully tested, $F_s\leq1.71$, $p_s\geq0.18$.

CGP 39551 d-CPPene, and Ro 25-6981 antagonism: Rather than reducing responding evoked by nicotine, some competitive NMDA receptor antagonists increased the nicotine-evoked CR (Fig. 1c). For CGP 39551 there was main effect of dose, $F(4,56)=3.58$, $p<0.05$; 10 mg/kg CGP 39551 evoked greater responding than nicotine alone. Similarly, for Ro 25-6981, there was a main effect of dose, $F(4,56)=3.60$, $p<0.05$; 1, 3, and 10 mg/kg Ro 25-6981 enhanced nicotine-evoked responding compared to nicotine alone. There was no effect of d-CPPene, $F(4,56)=1.64$, $p=0.18$. CGP 39551 and Ro 25-6981 had no significant effect on activity, $F_s\leq2.12$, $p_s\geq0.09$ (see Table 4). Although the ANOVA on activity for d-CPPene was significant, $F(4,56)=4.45$, $p<0.01$, the post-hoc comparisons did not indicate any significant differences.

NMDA and Ro 25-6981 substitution: Neither NMDA nor Ro 25-6981 substituted for the nicotine CS (Fig. 1d). Although the ANOVA was significant for NMDA, $F(3,39)=5.28$, $p<0.01$, the post-hoc comparisons did not reveal any significant differences. The ANOVA was not significant for Ro 25-6981, $F(3,36)=1.78$, $p=0.17$. As listed in Table 4, NMDA and Ro 25-6981 affected chamber activity. There was a main effect of NMDA, $F(3,39)=4.86$, $p<0.01$; 30 mg/kg NMDA reduced chamber activity compared to saline. Conversely, Ro 25-6981, $F(3,36)=6.80$, $p<0.001$, significantly enhanced activity at 10 mg/kg.

ACPC and L-701,324 antagonism: As shown in Fig. 1e, ACPC reduced nicotine-evoked conditioned responding. The ANOVA was significant, $F(3,39)=6.01$, $p<0.01$; dipper entries were reduced at 100 and 500 mg/kg but not at 300 mg/kg ACPC. Responding following pretreatment with 100 and 500 mg/kg was not decreased to the saline baseline ($M=0.029\pm0.004$) generated from the last saline training session before testing on 300 mg/kg ACPC, $t_s(13)\geq4.33$, $p<0.001$, indicating partial antagonism. There was no effect of ACPC on activity, $F<1$ (Table 4). L-701,324 reduced nicotine-evoked conditioned responding, but only at doses that also significantly decreased activity (data not shown). As such, we cannot interpret whether dipper entry reduction reflects antagonism of the CS effects of nicotine or general motor impairment.

Experiment 2a

Treatment with 0.2 mg/kg MK-801 reduced dipper entry rates regardless of whether it was given before nicotine or before saline (Fig. 2a). There was a main effect of drug state, $F(5,35)=5.15$, $p<0.001$. Post-hoc contrasts indicate that only MK-801 reduced conditioned responding compared to the Sal-Nic baseline (training) condition. Although the ANOVA on activity was significant, $F(3,35)=2.91$, $p<0.05$, there were no differences relative to the Sal-Nic condition (Table 5).

Experiment 2b

Discrimination training—Dipper entry rates were higher on saline than nicotine by the end of training, $t(6)=3.84$, $p=0.009$ ($M_s=0.266\pm0.06$ vs. 0.086 ± 0.01 on saline vs. nicotine sessions, respectively). There was also a difference in chamber activity, $t(6)=4.34$, $p=0.005$ ($M_s=0.307\pm0.05$ vs. 0.495 ± 0.02 on saline vs. nicotine sessions, respectively).

MK-801 antagonism—MK-801 dose-dependently decreased saline-appropriate responding (Fig. 2b). The one-way ANOVA was significant, $F(3,27)=5.33$, $p=0.008$. Subsequent contrasts indicating that 0.2 mg/kg MK-801 reduced the CR relative to saline alone to the level of the nicotine baseline ($M=0.096\pm0.02$) generated using the last nicotine training session for each rat before testing with 0.1 mg/kg MK-801 ($t<1$). The ED_{50} for MK-801 was 0.105 mg/kg. Table 5 shows that MK-801 dose-dependently increased chamber activity, $F(3,27)=9.81$, $p<0.001$, with the 0.2 mg/kg dose greater than saline treatment and reaching the level of the nicotine activity baseline, $M=0.529\pm0.05$, $t(6)=2.29$, $p=0.06$.

Discussion

The findings of the present report replicate and extend our previous research demonstrating the nicotine-evoked CR was dose-dependently blocked by MK-801 (Murray and Bevins 2007a). We found a similar effect in the present study with full antagonism of the nicotine (0.4 mg/kg)-evoked CR at 0.2 mg/kg MK-801. We extended these findings by testing PCP, another NMDA channel blocker, that has a slightly lower affinity for NMDA receptors than MK-801 (Wong et al. 1986) but still produces similar discriminative stimulus effects to MK-801 (e.g., Chaperon et al. 2003; Doty et al. 1994; Willetts and Balster 1988). PCP pretreatment (2 mg/kg) partially blocked the nicotine-evoked CR. However, no other ligand acting on the NMDA receptor fully blocked nicotine-evoked responding without profoundly altering motor activity (i.e., L-701,324). The only other consistent effect was an enhancement of nicotine-evoked conditioned responding when pretreated with Ro 25-6981, a highly selective antagonist of the NR2B receptor subtype (Fischer et al. 1997; Lynch et al. 2001; Mutel et al. 1998). In contrast, CGP 39551 and d-CPPene, specific but non-selective antagonists for NR2A/B NMDA receptor binding sites (Davies et al. 1986; Fagg et al. 1990; Lowe et al. 1994), had only minor effects on the CR. The enhanced responding found with

the high dose of CGP 39551 may reflect some preferential binding to the NR2B subtype over the NR2A subtype at the higher dose making it act similar to Ro 25,6981 (see Blaise et al. 2005).

A possible explanation for nicotine-enhanced responding with Ro 25-6981 is based on the observation that rats trained with 0.4 mg/kg nicotine can show an increase in responding when tested with 0.2 mg/kg in the DGT (Murray and Bevins 2007a) and operant drug discrimination (e.g., Pratt et al. 1983) tasks suggesting a possible performance-impairing effect of that higher nicotine dose. Although it is unclear what behavioral process may be affected (motor, appetite, etc.), the underlying mechanism could involve NR2B receptors. NR2B-containing receptors tend to be localized extrasynaptically and function to slow glutamatergic synaptic transmission in highly active synapses (Li et al. 2002; Massey et al. 2004; Stocca and Vicini 1998; Woodhall et al. 2001). Blocking these receptors can result in increased synaptic transmission, including transmission evoked by nicotine, which may in turn contribute to the enhanced behavioral output (CR) evoked by the nicotine CS. Although Ro 25-6981 increased responding to the nicotine CS, it did not substitute for nicotine when given alone, suggesting a lack of tonic NMDA activity in the absence of nicotine. Further, NMDA did not substitute for the nicotine CS, indicating that NMDA activation cannot be solely responsible for the nicotine-evoked CR regardless of the CR-attenuating effects of the channel blockers, MK-801 and PCP.

NMDA receptors have both NMDA (NR2) and glycine/D-serine (NR1) binding sites, and there is a general consensus that binding of both sites is required for NMDA receptor activation (e.g., Clements and Westbrook 1991; Kemp and Leeson 1993; Wolosker 2007). As glycine is acting as a co-agonist with NMDA and as it is often found in high tonic concentrations in vivo (Johnson and Ascher 1987), blocking this binding site should prevent NMDA receptor activation. As such, we tested L-701,324, a selective glycine antagonist at the NMDA receptor complex (Bristow et al. 1995; Rowley et al. 1993), to determine if the glycine binding site is the primary mechanism for the NMDA receptor component of the nicotine-evoked CR. Unfortunately, this compound decreased responding at doses that also attenuated locomotor activity making interpretation difficult. Nicotine co-administration with ACPC, a partial agonist at the NMDA receptor having agonist actions at the glycine/D-serine (NR1) binding site and antagonist actions at the NMDA (NR2) binding site (Nahum-Levy et al. 1999; Sheinin et al. 2002), should enhance the nicotine-evoked CR if there was a glycine component; attenuation of the CR should be seen if there was an NMDA component. We found some inconsistent partial antagonism with ACPC, suggesting again a possible NMDA role for the CS effects of nicotine.

Although the results from Experiment 1 hint at some role of NMDA activation in the CS effects of nicotine (cf. Ro 25-6981 and ACPC), there was no clear mechanism of the MK-801/PCP reduction in conditioned responding identified in that experiment. This outcome prompted us to examine whether reduction of the CR by MK-801 reflected a state-dependency effect. As detailed in the Introduction, there is extensive evidence that behaviors learned under the influence of MK-801 are not expressed in the absence of MK-801 (e.g., Schmidt et al. 1999). Further, and directly relevant to the current study, behaviors learned in the absence of MK-801 may no longer be expressed when tested in the presence of MK-801 (e.g., Wise et al. 1996). This state-dependency account would suggest that when we tested MK-801 pretreatment of the nicotine CS, MK-801 altered the state of the rats, preventing them from recalling the nicotine CS-sucrose US association and subsequently reducing their CR.

The results of Experiment 2a confirmed the predictions outlined in the Introduction. That is, removing nicotine or administering mecamylamine had no effect on goal-tracking

presumably because the chamber cues and not the nicotine state were evoking the CR—recall that sucrose and nicotine occurred on every session (cf., Bevins et al. 2007). In contrast, administration of MK-801 significantly reduced the goal-tracking CR without impacting locomotor activity. This disruption was seen whether saline or nicotine was also administered before the test, and is consistent with the suggestion that pretreatment with MK-801 prevented recall of the chamber CS-sucrose US association. By extension, MK-801 in Experiment 1 and in Murray and Bevins (2007a) may be preventing recall of the nicotine CS-sucrose US association in the DGT task rather than pharmacologically blocking the nicotine. Experiment 2b reversed the DGT contingencies so that sucrose was delivered on saline, but not on nicotine sessions. Thus, goal-tracking was high only on saline sessions; responding withheld on nicotine sessions. Pretreatment with MK-801 on saline test sessions dose-dependently reduced goal-tracking to the low level seen on nicotine sessions; the linear regression analyses used to establish ED_{50} s for each of the MK-801 dose effect curves were not different, $F(1,2)=4.77$, $p=0.16$. Combined, these results suggest that the effect of MK-801 in the DGT task was not specific to nicotine but instead disrupted discriminated goal-tracking responding by virtue of its state-changing effects.

The present results are especially interesting in light of previous research with nicotine as an operant discriminative stimulus. That research has shown that pretreatment with MK-801 and agmatine, another NMDA receptor antagonist (Yang and Reis 1999), did not affect nicotine-appropriate responding or overall response rates (Kim and Brioni 1995; Zakharova et al. 2005; Zaniewska et al. 2008). Importantly, the lack of effect on response rates in the operant literature indicates that motivation for the reinforcer was not affected—thereby suggesting the reduction in dipper entries in the current studies is unlikely due to reduced motivation for sucrose. Rather, there appears to be dissociation in the state-dependency effect of MK-801 in the DGT and two-lever drug discrimination tasks. Perhaps the explicit contingency between lever pressing and reinforcement delivery makes lever pressing more resistant to this effect of MK-801; dipper entries in the DGT task are not required for delivery of the reinforcer, nor does the frequency of head entries into the dipper change the rate of reinforcement. However, this explanation seems unlikely as Jackson et al. (1992) found that rats trained to press a single lever using food reinforcement in a saline state had responding severely disrupted when treated with MK-801 or PCP. Future research investigating the nature of the associative learning in these different tasks and their underlying mechanisms could suggest improved behavioral and pharmacological approaches to treating nicotine addiction (see Bevins 2009 and Wooters et al. 2009).

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Fig. 1.

Panel **a** shows dipper entry rate $\pm$ SEM for nicotine generalization tests. Panel **b** shows dipper entry rate $\pm$ SEM for antagonism tests with MK-801 and PCP. The *solid line* indicates the saline baseline dipper entry rate generated from the saline training session before each rat tested with 2 mg/kg PCP (*dotted lines* $\pm$ SEM). Panel **c** shows dipper entry rate $\pm$ SEM for antagonism testing with CGP 39551, d-CPPene, and Ro 25-6981. Panel **d** shows dipper entry rate $\pm$ SEM for substitution testing with NMDA and Ro 25-6981. Panel **e** shows dipper entry rate $\pm$ SEM of antagonism testing with ACPC. The *solid line* indicates saline baseline dipper entry rate generated from the saline training session before each rat tested with 300 mg/kg ACPC (*dotted lines* $\pm$ SEM). For all panels, # indicates difference from vehicle baseline, $p < 0.05$; ## $p < 0.01$; ### $p < 0.001$. * indicates difference from vehicle pretreatment of nicotine or nicotine alone, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ^ indicates main effect of test drug in the absence of significant post-hoc comparisons

Fig. 2.

Panel **a** shows dipper entry rate $\pm$ SEM of the state-change testing in Experiment 2a. Panel **b** shows dipper entry rate $\pm$ SEM of antagonism testing with MK-801 of the saline CS in Experiment 2b. The *solid line* indicates nicotine baseline dipper entry rate generated from the nicotine training session before each rat tested with 0.1 mg/kg MK-801 (*dotted lines* $\pm$ SEM). For both panels, * indicates difference from vehicle pretreatment of nicotine or nicotine alone, $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Drug preparation and administration details

Table 1

Compound	Vehicle	Route	Volume (ml/kg)	IP ^a (min)	Reference
Nicotine	Saline, pH to 7.0±0.2 with NaOH	SC ^b	1	5	Besheer et al. 2004; Murray and Bevins, 2007a
MK-801	Saline	IP ^c	1	30	Doty et al. 1994
PCP	Saline	IP	1	30	Doty et al. 1994
CGP 39551	Distilled water	IP	2	30	Kosowski et al. 2004
d-CPPene	Distilled water	IP	2	30	Shoaib et al. 1994
Ro 25-6981	Distilled water	IP	2	30	Chaparon et al. 2003; Konitsiotis et al. 2006
NMDA	Distilled water	IP	1	10	Grech et al. 1993
L-701,324	DMSO	IP	0.5	30	Hundt et al. 1998
ACPC	Distilled water	IP	2	30	Papp et al. 2002
Mecamylamine	Saline	IP	1	30	Glick et al. 2002; Struthers et al. 2009

^a Injection-to-placement interval
^b Subcutaneous
^c Intraperitoneal

Table 2

Antagonism and substitution testing phases in Experiment 1 (Nicotine CS)

Test type ^a	Injection 1	Doses (mg/kg)	Injection 2	Doses (mg/kg)	Subjects ^b (N)
Generalization	–	–	Nicotine	Saline, 0.025, 0.05, 0.1, 0.2, 0.4	16
Antagonism	MK-801	0.03, 0.1, 0.2	Nicotine	0.4	15
	PCP	0.3, 1, 2	Nicotine	0.4	
	Saline ^c	–	Nicotine	0.4	
Antagonism	CGP 39551	0.3, 1, 3, 10	Nicotine	0.4	15
	d-CPPene	0.3, 1, 2, 3	Nicotine	0.4	
	Ro 25-6981	1, 3, 10, 20	Nicotine	0.4	
	Water ^c	–	Nicotine	0.4	
Substitution	NMDA	3, 10, 30	–	–	14
	Water	–	–	–	
Antagonism	L-701,324	0.1, 0.3, 1, 3	Nicotine	0.4	14
	DMSO	–	Nicotine	0.4	
Antagonism	ACPC	100, 300, 500	Nicotine	–	13
	Water	–	Nicotine	–	
Substitution	Ro 25-6981	1, 3, 10	–	–	13
	Water	–	–	–	

^a All tests within each phase are randomly intermixed. One phase is completed before the next begins

^b Consistent failure to meet the discrimination criterion resulted in removal of subject from the remainder of the experiment

^c Vehicle baseline used for all drugs tested within a phase

Table 3

Tests in Experiment 2a (State change)

Injection 1 ^a	IPI ^b (min)	Route	Injection 2	IPI	Route
Saline	30	IP ^c	0.4 mg/kg nicotine	5	SC ^d
Saline	30	IP	Saline	5	SC
0.2 mg/kg MK-801	30	IP	0.4 mg/kg nicotine	5	SC
0.2 mg/kg MK-801	30	IP	Saline	5	SC
1 mg/kg mecamylamine	30	IP	0.4 mg/kg nicotine	5	SC
1 mg/kg mecamylamine	30	IP	Saline	5	SC

^aAll tests are randomly intermixed

^bInjection-to-placement interval

^cIntraperitoneal

^dSubcutaneous

Table 4Activity data ($\pm$ SEM) for Experiment 1 (Nicotine CS) in beam breaks per second

	Nicotine ^a	Vehicle	Dose (mg/ml)			
Nicotine ^b	0.38 $\pm$ 0.03	0.32 $\pm$ 0.03	0.025	0.05	0.1	0.2
MK-801 ^c	0.40 $\pm$ 0.04	–	0.34 $\pm$ 0.03	0.37 $\pm$ 0.04	0.43 $\pm$ 0.4 [*]	0.45 $\pm$ 0.04 ^{**}
			0.03	0.1		0.2
PCP ^c	0.40 $\pm$ 0.04	–	0.40 $\pm$ 0.04	0.37 $\pm$ 0.04		0.35 $\pm$ 0.03
			0.3	1	2	
CGP 39551 ^c	0.36 $\pm$ 0.04	–	0.41 $\pm$ 0.04	0.37 $\pm$ 0.02		0.32 $\pm$ 0.03
			0.3	1	3	10
Ro 25-6981 ^c	0.36 $\pm$ 0.04	–	0.35 $\pm$ 0.04	0.36 $\pm$ 0.04	0.41 $\pm$ 0.06	0.41 $\pm$ 0.03
			1	3	10	20
d-CPPene ^c	0.36 $\pm$ 0.04	–	0.43 $\pm$ 0.03	0.48 $\pm$ 0.05	0.46 $\pm$ 0.04	0.39 $\pm$ 0.04
			0.3	1	2	3
NMDA ^e	–	0.28 $\pm$ 0.04	0.43 $\pm$ 0.03	0.33 $\pm$ 0.04	0.32 $\pm$ 0.03	0.27 $\pm$ 0.04 ^d
			3	10	30	
Ro 25-6981 ^e	–	0.28 $\pm$ 0.03	0.29 $\pm$ 0.03	0.25 $\pm$ 0.04		0.11 $\pm$ 0.03 ^{**}
			1	3	10	
ACPC ^c	0.32 $\pm$ 0.02	–	0.31 $\pm$ 0.04	0.31 $\pm$ 0.03		0.46 $\pm$ 0.03 ^{***}
			100	300	500	
			0.34 $\pm$ 0.02	0.35 $\pm$ 0.03		0.31 $\pm$ 0.03

*
 $p < 0.05$,**
 $p < 0.01$,***
 $p < 0.001$; indicates difference from vehicle baseline^aNicotine (0.4 mg/kg) when given alone or with the 0 mg/kg drug pretreatment^bGeneralization^cAntagonism

Indicates main effect of drug dose in the absence of significant post-hoc comparisons
p
e Substitution

Table 5

Activity data ($\pm$ SEM) for Experiments 2a and 2b in beam breaks per second

State change	Sal-Nic	Sal-Sal	0.2 MK-Nic	0.2 MK-Sal	1 Mec-Nic	1 Mec-Sal
	0.36 $\pm$ 0.04	0.29 $\pm$ 0.02	0.28 $\pm$ 0.04	0.46 $\pm$ 0.09	0.31 $\pm$ 0.04	0.27 $\pm$ 0.03 ^a
	Saline ^b	Dose (mg/ml)				
MK-801	0	0.03		0.1		0.2
	0.29 $\pm$ 0.06	0.32 $\pm$ 0.06		0.47 $\pm$ 0.04		0.68 $\pm$ 0.05 ^c

^aIndicates main effect of drug dose in the absence of significant post-hoc comparisons

^bSaline CS when given along with the 0 mg/kg MK-801 pretreatment

^cIndicates difference from vehicle pretreatment of the saline baseline, $p < 0.001$, and equivalence to nicotine activity baseline