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Competitive and noncompetitive NMDA antagonist effects in rats trained to discriminate lever-press counts

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Abstract

The glutamate activated *N*-methyl-D-aspartate (NMDA) receptor may play a role in short-term memory processing. Among the evidence for this is that NMDA antagonists can impair accuracy in fixed consecutive number (FCN) tasks. This study was designed to further characterize this effect by examining NMDA antagonists differing in their cellular mechanisms of action. Rats were trained to respond under an FCN operant schedule, which required eight presses on one lever (counting lever) before one press at an alternate lever (reinforcement lever) would produce food reinforcement. The effects of three noncompetitive [MK-801 (0.01–0.56 mg/kg); phencyclidine (0.3–3.0 mg/kg); memantine (1–10 mg/kg)] and two competitive [SDZ EAA 494 (0.3–3.0 mg/kg) and NPC 17742 (2.0–16 mg/kg)] NMDA antagonists were analyzed. MK-801 and phencyclidine decreased accuracy at doses not reducing response rates. Memantine, and both of the competitive antagonists, also reduced accuracy, but did so only at doses that markedly reduced response rates. These results suggest that both the affinity and the site bound on the NMDA glutamate receptor by antagonists can determine their effects on FCN performance. Subsequent studies investigated whether SCH 23390, a dopamine D1 receptor antagonist, and NMDA could modulate the effects by phencyclidine and SDZ EAA 494, respectively, on FCN performance.

Keywords: Fixed consecutive number; Complex discrimination; Counting behavior; Working memory; NMDA receptor; Competitive antagonist; Noncompetitive antagonist; Rat

1. Introduction

N-methyl-D-aspartate (NMDA) receptors are ligand-gated ion channel complexes with several binding sites for modulating ionic flux. Selective antagonists can attenuate neurotransmission by binding to the transmitter (glutamate) recognition site, to the co-transmitter (glycine) recognition site, to a site within the ion channel or to polyamine modulatory sites. Recent years have seen considerable interest in studies designed to elucidate the modulatory functions of NMDA receptors in learning and memory processes.

One way to characterize the role of the NMDA receptor in short-term memory is to study behavior that is thought to involve short-term memory and then manipulate NMDA receptor-mediated neurotransmission using pharmacologic agents that bind selectively to its modulatory centers. NMDA antagonist compounds have been tested in a variety of species and results indicate their ability to disrupt performance in memory-sensitive behavioral tasks. This has been demonstrated in studies involving maze navigation (Morris et al., 1986, 1989; Murray, 1995), delayed matching to sample (Karbon et al., 1992; Deacon and Rawlins, 1995; Doyle et al., 1998; Miller and Desimone, 1994; Stanhope et al., 1995), time discrimination (Berz et al., 1992; Paule, 1994) and repeated acquisition (Cohn et al., 1992; Moerschbaecher and Thompson, 1980). Understudied have been the effects of NMDA antagonists on counting behavior. Counting behavior can be assessed in laboratory animals by using an operant conditioning schedule that requires the subject to discriminate the number of lever-presses emitted (count discrimination tasks), or to discriminate cycles of lever-

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press sequences (targeted percentile or cyclic ratio tasks). Counting free operant schedules has been routinely used to assess memory impairment (Boysen et al., 1995; Capaldi and Miller, 1988; Davis and Perusse, 1988; Gibbon et al., 1997), but only three of many available noncompetitive NMDA antagonist compounds have been tested using this procedural design, and no competitive agents have been tested.

The study described here compared the effects of competitive NMDA antagonists, which bind at the same site as the endogenous transmitter, to noncompetitive antagonists, which bind at intrachannel sites, using a ratio-based count discrimination procedure called the fixed consecutive number (FCN) procedure. FCN procedures require a subject to emit a specified number of responses on one lever (counting lever) before a response at another lever (reinforcement lever) results in reinforcement. Performances under FCN operant schedules are disrupted by treatments that presumably impair short-term memory (Doty et al., 1992; Idrobo et al., 1988; Vidyasagar, 1996). Because dopamine neurotransmission is influenced by acute and chronic administrations of channel-blocking NMDA antagonist compounds (Espaze et al., 2000; He et al., 1993), a rationale existed to assess the pharmacologic specificity of the NMDA antagonist-induced accuracy disruptions we observed. We thus evaluated the effects of SCH 23390, a dopamine D1 receptor antagonist, on the effects of PCP on FCN performance. Antagonism of D1 receptors was specifically explored because a very dense population of D1 receptors exists in cortical regions of the brain where short-term memory is putatively mediated (El-Ghundi et al., 1999; Goldman-Rakic, 1992; Smith et al., 1998). Additionally, we co-administered the selective agonist, NMDA, with the competitive NMDA antagonist, SDZ EAA 494, to help determine whether NMDA receptors were the principle mediators of these accuracy-disrupting effects.

2. Methods

2.1. Subjects

Twelve rats were used as subjects for evaluating the effects of test compounds on FCN performance. Two strains of rats were used in these studies as an intermediate step before the laboratory chose, and committed to using a single, common strain. Subjects 4-01, 4-02, 4-03, 4-04, 4-09, 4-11, 4-12, 4-13, 4-17 were male Long–Evans hooded rats (360–384 g; Harlan Laboratories, Dublin, VA). The remaining three rats, numbered 4-05, 4-06 and 4-07, were male Sprague–Dawley rats (340–430 g; COBS, Charles River, Wilmington, MS). All rats were approximately 3 months of age at the start of the studies. The rats were individually housed with ad libitum access to water in a colony room maintained at 20–22°C. The rats were maintained at approximately 85% of their free-fed weight with supplemental feedings of Purina Rodent Chow immediately after daily testing or training sessions. Seven Long–Evans rats were used in interaction studies entailing the co-administration of (i) PCP with SCH 23390 and (ii) SDZ EAA 494 (D-CPPene) with NMDA. All experiments were conducted during the light period of a 12/12 h day–night cycle (08:00–20:00 h).

2.2. Apparatus

Six standard two-lever operant conditioning chambers (BRS/LVE, Beltsville, MD) were connected to a 486/66 MHz microcomputer through an interface and controlled by MED-PC software (MED Associates, East Fairfield, VT). The chambers were equipped with a bank of three (white) stimulus lights above the response levers, a house light and a food dispenser, which delivered 45-mg food pellets (PJ Noyes, Lancaster, NH). Left and right response levers were calibrated similarly in each of the six operant chambers and required a force in excess of 0.2 N to produce switch closure.

3. Procedure

The beginning of each experimental session was signaled by the illumination of the stimulus lights above the response levers. Rats were initially trained to lever-press for food pellet delivery according to fixed consecutive number-1 (FCN-1) schedule of reinforcement. Contingencies for preliminary training under an FCN-1 schedule required at least one press of the ‘counting’ lever (left side lever for rats 4-01, 4-02, 4-05, 4-06, 4-09, 4-11; right side lever for rats 4-03, 4-04 and 4-07, 4-12, 4-13) followed by a single press of the ‘reinforcement’ lever to produce food pellet delivery. The minimum response requirement for the counting lever was gradually increased to eight. With each gradual increase, a reset contingency for premature lever switching was in effect. Responding fewer than eight times on the counting lever before pressing the reinforcement lever was recorded as an ‘early mistake’ and reset the response requirement. Responding more than 14 times on the counting lever before switching to the reinforcement lever was recorded as a ‘late mistake,’ but did not reset the response requirement, except in the testing of memantine. The procedure was changed prior to testing memantine, and for the interaction studies, in recognition that late mistakes had rarely occurred during tests with earlier drugs and to minimize the adventitious reinforcement of long runs of counting responses. Cue lights above each response lever were lit throughout sessions, as was the central houselight.

Any lever-press sequence, which included a response on the counting lever followed by a response on the

reinforcement lever, was recorded as a 'switch.' All training and testing sessions were 30 min in duration and were held daily (Monday–Sunday). Daily training for each subject continued until stable and accurate responding was displayed. Test compounds were not administered unless the following stability requirements were met: (i) the percentage of reinforced lever switches (i.e., accuracy) on the day before testing must not have varied by more than 10% of the mean value for accuracy across five preceding vehicle sessions; and (ii) response rate on the day before testing must not have varied by more than 10% from the mean response rate across five preceding vehicle sessions. Rats meeting these criteria were tested with drug compounds approximately twice per week.

The dose ranges and pre-session injection times for determining drug effects on schedule-controlled responding were: PCP (0.056–4 mg/kg; pre-session injection time 15 min), MK-801 (0.03–0.2 mg/kg; pre-session injection time 15 min), memantine (1–10 mg/kg; pre-session injection time 30 min), SDZ EAA 494 (1–3 mg/kg; pre-session injection time 60 min), NPC 17742 (2–16 mg/kg; pre-session injection time 60 min), and were chosen based upon literature values. A minimum of four subjects was used for each dose–effect determination. The order of testing for single drug treatments was: PCP ($n=4$), SDZ EAA 494 ($n=7$), MK-801 ($n=4$), NPC 17742 ($n=4$) and memantine ($n=6$). Doses were administered in random order for all experiments.

Drug–drug interaction studies were additionally conducted to help determine the specificity of the effects exerted by some of the antagonists. PCP was co-administrated with the selective D1 dopamine receptor antagonist, SCH 23390, and SDZ EAA 494 was co-administered with the selective agonist, NMDA. In the SCH 23390/PCP combination study, the dose of SCH 23390 was held constant at 0.03 mg/kg. This dose of SCH 23390 was chosen because it was the highest dose that did not produce significant effects on FCN performance by itself during preliminary dose–response tests, which ranged from 0.01 to 0.1 mg/kg. SCH 23390 (0.03 mg/kg) was administered 30 min, and PCP (0.56–2 mg/kg) 15 min prior to session start. In the NMDA/SDZ EAA 494 combination study, the dose of NMDA was held constant at 17 mg/kg. This dose was chosen because it did not produce significant effects on FCN performance by itself during preliminary dose–response tests, which ranged from 10 to 30 mg/kg. SDZ EAA 494 (1–3 mg/kg) was administered 60 min, and NMDA (17 mg/kg) 10 min prior to session start.

3.1. Drugs

Dose–effect functions for five NMDA antagonists were determined. Phencyclidine (PCP; National Institute on Drug Abuse, Rockville, MD), dizocilpine (MK-801; National Institute on Drug Abuse) and memantine (provided by C. Parsons, Frankfurt, Germany) were dissolved in physiological saline. NPC 17742 (Nova Pharmaceuticals, Baltimore, MD) and SDZ EAA 494 (provided by Novartis Pharma, Basel, Switzerland) were solubilized in equimolar NaOH and brought to volume with saline. For interaction studies, SDZ EAA 494 and PCP were prepared as above, while NMDA (Sigma Chemical, St. Louis, MO) was dissolved in equimolar sodium hydroxide and SCH 23390 [(*R*)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol] (Sigma) was dissolved in water. Each drug was injected in a volume equivalent to 1 ml/kg body weight. Vehicle injections, by identical routes to their respective test drugs, were administered prior to every training session.

3.2. Data analysis

The data collected from each experimental session included: (i) accuracy measure–percentage of reinforced runs [(number of pellet deliveries)/(number of pellet deliveries + number of nonreinforced runs) × 100]; and (ii) rate measure–response rate (total number of responses on both levers/session duration). Reinforced run calculations reflect 'correct' response chains emitted in the fixed length testing sessions. The reinforced run calculations were made after excluding data for rats that emitted fewer than 180 total lever presses in a test session. The rationale for this exclusion was to minimize inferences based upon severely intoxicated performances. The rate calculations included all test session data. Effects were further analyzed by calculating ED50 values with 95% confidence limits according to a modification of Procedures 5 and 8 by Tallerida and Murray (1987), which was originally advocated by Colquhoun (Colquhoun, 1971). Tests for parallelism were conducted before calculation of potency ratio values and 95% confidence limits using the method of Colquhoun. The curves generated by these data were parallel enough to permit use of the Colquhoun potency ratio algorithm. The Colquhoun method utilizes a statistically validated algorithm to express potency in relative terms by evaluating the density and spread of two scatter plot dispersions. In applying Colquhoun's methodologies to this dataset, the $\log_{10}$ transformed dose and the measured effect in each subject were analyzed as dispersed datapoints. For the purpose of examining accuracy relative to baseline accuracy, raw data were transformed prior to Colquhoun ED50 analyses using the following equation, $[(\%\ \text{Runs Reinforced})_{\text{test session}}/(\%\ \text{Runs Reinforced})_{\text{vehicle control session}}]$, at each dosing level. Thus, the calculated ED50 values reflect potencies for changing typical control session performances and, importantly, these ED50 values do not reflect potencies for changing accuracy relative to the theoretical ideal (100% Runs Reinforced). Potency differences were considered significant if no overlap existed in ED50 confidence ranges for accuracy and rate measures.

4. Results

4.1. FCN performance effects: single drug testing sessions

There was no more than 10% variation from the mean baseline 'accuracy' for each individual rat during training sessions prior to testing, indicating that the subjects had acquired the task and stabilized within this range prior to testing. The results for tests with MK-801 are shown in the upper panel of Fig. 1. Two dosages of MK-801 were shown to produce large deficits in performance accuracy, and accuracy was decreased without a concomitant rate reduction. Fig. 1 shows that performance in sessions with presession administration of 0.03 and 0.056 mg/kg MK-801 was less accurate, but there was no significant change in response rate. The selective effect that MK-801 had on accuracy is also apparent when examining the ED50 values for each measure (Table 1). MK-801's potency for disrupting accuracy was larger than its potency for reducing response rate (ratio 0.53), and the two effects were statistically separable.

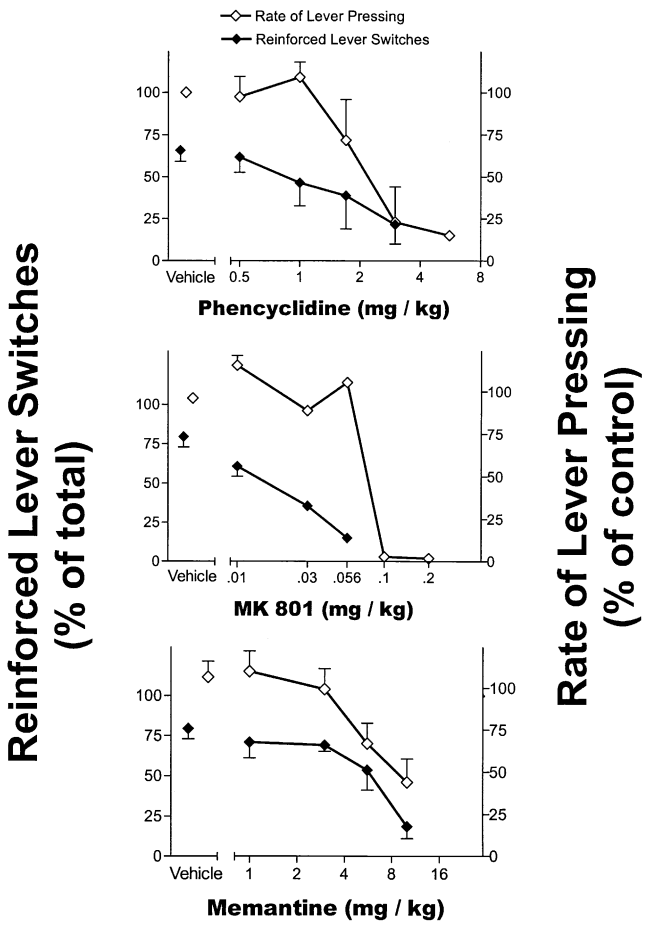

Fig. 1. Effects of noncompetitive NMDA antagonists on the accuracy and rate of FCN schedule-controlled responses. The upper panel shows results from tests with phencyclidine (PCP). The central panel shows results from tests with dizocilpine (MK-801). The lower panel shows results from tests with memantine. Filled data points indicate the group mean percentage of reinforced lever switches. Open data points indicate the group mean percentage of control session response rates. Error bars display standard error of the mean.

The accuracy and rate effects determined for various doses of PCP are shown in the center panel of Fig. 1. PCP began to lower the accuracy of FCN responses before it lowered response rates. In sessions where a 1.7 mg/kg dose of PCP had been injected prior to behavioral testing, there was an approximate 50% drop in discrimination accuracy, while rate changed by only 25%. In testing sessions with the 1.0 mg/kg dose, performance of the group appeared less accurate, but the rate of responding remained close to the baseline level. Although the plotted data are suggestive of some selectivity for disrupting accuracy over rate, the effect did not achieve significance (e.g., see confidence limits on the ED50 values for accuracy and rate in Table 1). The potency ratio for reducing discriminative accuracy relative to rate equaled 0.74 (Table 1), and the graphed data indicate a higher propensity for PCP to disrupt accuracy than to limit the rate of lever-pressing.

The results for tests of the low-affinity uncompetitive NMDA antagonist compound, memantine, are shown in the lower panel of Fig. 1. The form of the dose–effect curve suggests that memantine compromised FCN accuracy (e.g., view performance in sessions testing the 10 mg/kg dose of memantine). The accuracy impairment, however, was accompanied by a large decrease in the rate of responding. The ED50 values indicated a higher propensity for memantine to affect response rate than to alter accuracy (Table 1). The ratio relating memantine's effects on each behavioral measure equaled 1.40.

The results obtained when testing two compounds that compete for the glutamate recognition site on the NMDA ion channel complex are shown in Fig. 2. The upper panel in Fig. 2 shows accuracy and rate effects for SDZ EAA 494. Although the accuracy of FCN performance in this group of trained subjects decreased for several doses of SDZ EAA 494, there was a concomitant decrease in the rate of FCN responding. The 3.0 mg/kg dose of SDZ EAA 494 impaired performance to such an extent that the percent of reinforced trials fell to zero. However, the 3.0 mg/kg dose simultaneously reduced the rate of responding, meaning that drug effects on accuracy were not selective. The $ED50_{(accuracy\ impairment)}$ for SDZ EAA 494 could not be determined (Table 1) since it exceeded the highest dose tested in this study — 3.0 mg/kg. The values determined in the ED50 analysis were most likely skewed by nonlinearity of the function relating accuracy to SDZ EAA 494 dose. In SDZ EAA 494 testing sessions, variable effects were noted in individual animals. Single subject data for rats injected with SDZ EAA 494 indicated an 'all or none' type of susceptibility to the accuracy compromising effects of SDZ EAA 494. There was a wide dispersion of individual SDZ EAA 494 session-derived datapoints, and this weakens the

Table 1
Effects of noncompetitive and competitive NMDA antagonists on accuracy and response rate for rats responding under an FCN schedule

Treatment	Task accuracy ED50 in mg/kg (CL)	Rate of responding ED50 in mg/kg (CL)	Potency ratio (accuracy/rate)[a]
Noncompetitive antagonists			
MK-801	0.03 (0.02, 0.04)	0.06 (0.04, 0.10)	0.53*
PCP	1.67 (0.96, 2.94)	2.42 (1.34, 4.35)	0.74
Memantine	7.10 (4.20, 12.0)	4.77 (3.35, 6.79)	1.40
Competitive antagonists			
SDZ EAA 494	>3.02[b]	1.04 (0.59, 1.83)	Indeterminate
NPC 17742	5.09 (3.84, 6.73)	5.32 (3.87, 7.30)	0.83

[a] Calculated by the method of Colquhoun (1971).
[b] Value exceeds highest (3.0 mg/kg) dose.
* Accuracy and rate differ statistically ($P < .05$).

reliability of the $ED50_{(accuracy\ impairment)}$. With this caveat, it should be noted SDZ EAA 494 was more potent for reducing response rates in FCN testing sessions than it was for reducing accuracy (Table 1).

The lower panel of Fig. 2 shows results for tests with NPC 17742. This glutamate site-selective NMDA antagonist also lowered accuracy in a nonselective manner. Pre-session injection of a 6 mg/kg dose of NPC 17742 disrupted response patterns, and FCN contingencies were satisfied for a mean of only 23% of lever switches in the session. The same dose, however, substantially decreased the rate of FCN responding. The accuracy effects of NPC 17742 were not selective, and this is reflected by the ED50 values determined for accuracy and rate (Table 1).

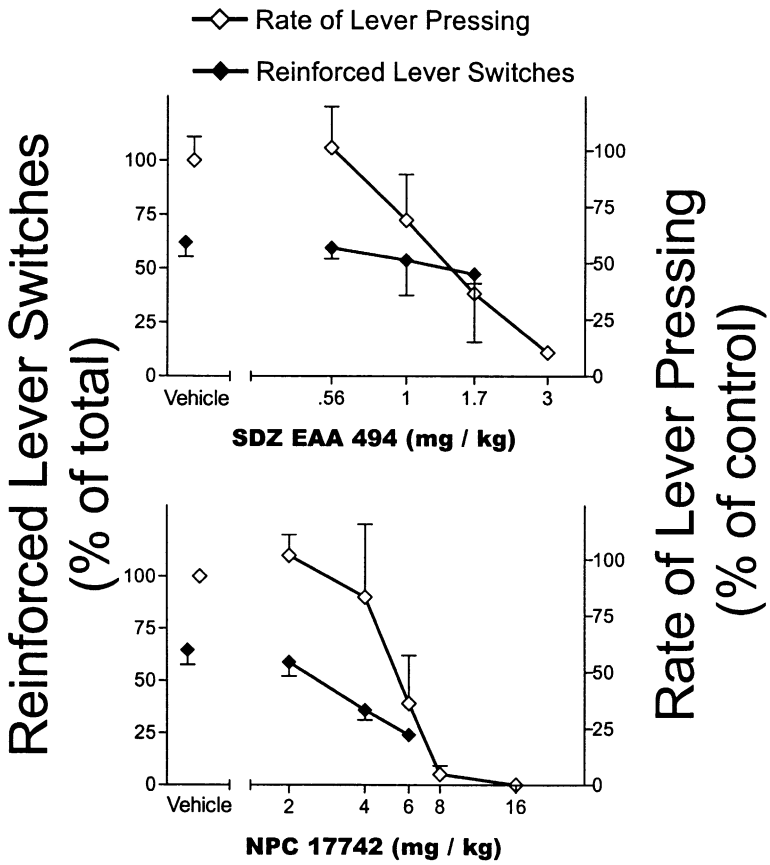

Fig. 2. Effects of competitive NMDA antagonists on the accuracy and rate of FCN schedule-controlled responses. The upper panel shows results from tests with D-CPPene (SDZ EAA 494). The lower panel shows results from tests with NPC 17742. Filled data points indicate group mean percentage of reinforced lever switches. Open data points indicate the group mean percentage of control session response rates. Error bars display standard error of the mean.

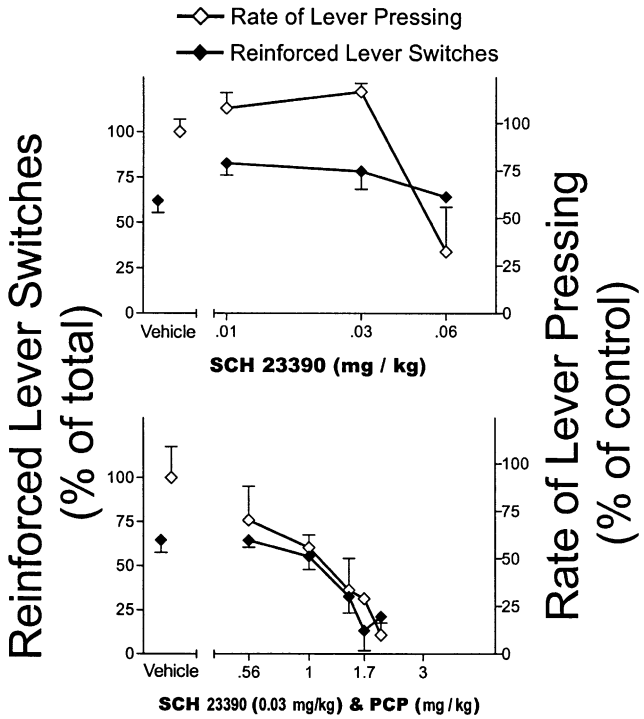

Fig. 3. Upper panel shows the effects of SCH 23390 on the accuracy and rate of FCN schedule-controlled responses when injected alone. The lower panel shows effects from co-administering a 0.03 mg/kg dose of SCH 23390 with various doses of phencyclidine (PCP). Filled data points indicate the group mean percentage of reinforced lever switches. Open data points indicate the group mean percentage of control session response rates. Error bars display standard error of the mean.

4.2. FCN performance effects: interaction tests

Fig. 3 shows results obtained in the PCP/SCH 23390 interaction experiments. The upper panel of Fig. 3 shows that SCH 23390, when injected alone, did not alter accuracy. The same graph shows that the mean response rate among tested animals varied slightly from control levels when SCH 23390 was injected prior to behavioral testing.

The lower panel of Fig. 3 shows accuracy and rate results when PCP was administered along with the SCH 23390. When given together, these drugs reduced response rates. The $ED50_{(rate\ reduction)}$ for PCP alone was 2.42; while the $ED50_{(rate\ reduction)}$ for PCP when co-administered with SCH 23390 was 0.97 (Table 2). Less change was induced in FCN accuracy. The $ED50_{(accuracy\ impairment)}$ was 1.67 mg/kg for PCP alone; while the value for PCP co-administered with SCH 23390 was calculated to equal 1.27 mg/kg (Table 2).

Fig. 4 shows results obtained during the NMDA/SDZ EAA 494 interaction tests. The upper panel of Fig. 4 shows that NMDA, when injected separately, did not affect the accuracy of FCN schedule-controlled responses. At the highest administered dose (30 mg/kg), NMDA suppressed the rate of lever-pressing. The lower panel of Fig. 4 shows results obtained when doses of SDZ EAA 494 were co-administered with 17 mg/kg NMDA. The highest co-administered dose of SDZ EAA 494 (3 mg/kg) impaired performance to such an extent that the percentage of reinforced runs fell to 27%. This result can be compared to performance in the session testing a singular dose of SDZ EAA 494 at 3 mg/kg. When administered alone, the 3.0 mg/kg dose of SDZ EAA 494 produced FCN response chains that were 100% inaccurate and resulted in zero food pellet deliveries. Co-administration of SDZ EAA 494 with 17 mg/kg of the selective agonist NMDA partially reversed accuracy effects produced by the former compound when it was injected alone. The reversal of accuracy effects is also reflected by a shift in the $ED50_{(accuracy\ impairment)}$ values under singular and paired treatment conditions (Table 2). Less of a shift in the derived ED50 values is noted for the response rate measure

Table 2
Interactive effects of PCP and SDZ EAA 494 with modulators dopaminergic/NMDA receptor neurotransmission

Treatment	Task accuracy ED50 in mg/kg (CL)	Rate of responding ED50 in mg/kg (CL)	Potency ratio (accuracy/rate)[a]
NMDA antagonists alone			
PCP	1.67 (0.96, 2.94)	2.42 (1.34, 4.35)	0.74
SDZ EAA 494	>3.0 mg/kg[b]	1.04 (0.59, 1.83)	Indeterminate
Competing ligands alone			
SCH 23390	Indeterminate	21.52 (0.02, 28,236.0)	Indeterminate
NMDA	Indeterminate	10.0 (4.87, 20.60)	Indeterminate
NMDA/D1 ligand interactions			
PCP/SCH 23390	1.27 (1.0, 1.62)	0.97 (0.76, 1.23)	0.75
Dual treatment[c]	4.94 (0.26, 93.23)	1.40 (0.97, 2.01)	0.67

[a] Calculated by the method of Colquhoun (1971).
[b] Value exceeds highest (3.0 mg/kg) dose.
[c] SDZ EAA 494 and NMDA co-administered.

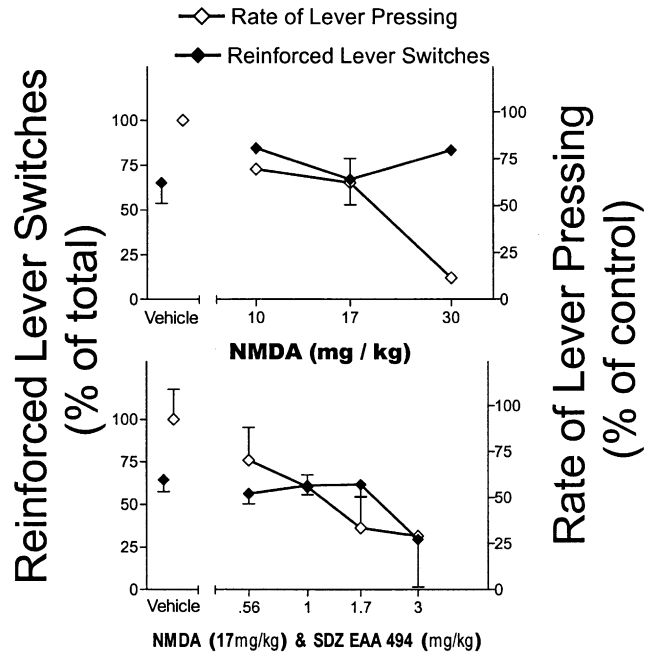

Fig. 4. Upper panel shows the effects of NMDA on the accuracy and rate of FCN schedule-controlled responses when injected alone. The lower panel shows effects from co-administering a 17 mg/kg dose of NMDA with various doses of D-CPPene (SDZ EAA 494). Filled data points indicate the group mean percentage of reinforced lever switches. Open data points indicate the group mean percentage of control session response rates. Error bars display standard error of the mean.

under singular vs. paired treatment conditions. An examination of response rate effects revealed that the 3 mg/kg dose of SDZ EAA 494, whether injected singularly or in combination, reduced lever-pressing rates to near zero.

5. Discussion

FCN studies involving the evaluation of putative amnestic agents, such as scopolamine (Laties, 1972; Vidyasagar, 1996), benzodiazepines (Evenden, 1998) and Δ9-THC (Mansbach et al., 1996), have reported disruptions of accuracy at doses that do not depress response rates. The present study was undertaken to analyze changes in FCN performance after pharmacologically attenuating NMDA neurotransmission. These results indicate that both competitive and noncompetitive NMDA receptor antagonists can impair FCN performance; however, only the noncompetitive antagonists did so without also impairing response rates. In so far as FCN performance can be construed as entailing working memory, these results are consistent with other studies reporting NMDA antagonist-induced disruption of working memory (Picker et al., 1987; Sanger, 1993; van Haaren et al., 1989).

The function of the NMDA receptor with regard to learning and memory processes has evoked considerable controversy. Many investigators hypothesize a major role for the NMDA receptor in acquisition (Morris et al., 1986; Shapiro and Caramanos, 1990), but a more limited role for recalling information, as would be necessary to perform a well-learned task. In the present studies, all antagonists potently decreased the accuracy of count discrimination in well-trained subjects. This result adds to and extends evidence gained by other methods suggesting a role for the NMDA receptor in recalling and performing established behaviors (Deacon and Rawlins, 1995; Lyford et al., 1993; Pontecorvo et al., 1991).

A comparison of the results obtained for competitive and noncompetitive NMDA antagonists reveals similar, as well as qualitatively different, effects. Competitive and channel-blocking antagonists have been evaluated in other laboratories, and commonalties and differences have been observed (Liljequist et al., 1991; Maj et al., 1991; Mansbach and Balster, 1991; Meldrum, 1985). Differences were noted in the ability of competitive and noncompetitive antagonists to produce selective memory effects. PCP and MK-801 results might be interpretable as 'mnemonic impairment' because changes in accuracy were not accompanied by response rate effects. The third noncompetitive antagonist, memantine, has lower affinity for the intrachannel site (Chen and Lipton, 1997; Mealing et al., 1999) and has unusual binding kinetics (Blanpied et al., 1997; Sobolevsky et al., 1998). The accuracy effects in memantine tests were not dissociable from response rate effects. Therefore, accuracy changes cannot be unambiguously attributed to memory disruption, and this suggests that specific dynamics of the interaction at NMDA neuromodulating centers is important for predicting behavioral effects. With competitive antagonists, the potency to disrupt counting behavior matched the potency for response suppression (Table 1), again complicating and limiting the conclusions that can be drawn. Conflicting evidence exists in the literature from studies that have evaluated memory after treatment with glutamate site-selective NMDA antagonist compounds. Early studies reported a selective impairment in short-term memory (Cole et al., 1993; Pontecorvo et al., 1991; van Haaren et al., 1989), but more recent work has indicated normal mnemonic function after treatment with SDZ EAA 494 (Ballard and McAllister, 2000).

Competitive NMDA antagonists have poor access to and poor distribution within the CNS (Boddeke et al., 1992; Chapman et al., 1982; Croucher et al., 1982), and this has made them a difficult group of compounds to characterize behaviorally. Even when administered directly into cerebral ventricles, competitive ligands may not gain access to more distal brain sites (O'Neill and Liebman, 1987). Furthermore, pharmacokinetic assays have indicated that the bioavailability of competitive antagonists is time-dependent (Boddeke et al., 1992; Nehls et al., 1988). Because the physiological substrates for short-term memory exist predominantly in cortical regions of the brain (Fuster, 1997; McCarthy et al., 1997; Quintana and Fuster, 1993), inferences made about the effects of drugs on short-term memory are strongest

when the drugs have known cortical penetration. Since pharmacokinetic difficulties have been reported, a potential limitation for studies involving peripherally injected competitive NMDA antagonists is their questionable access to receptors of interest. Thus, the present results are best interpreted in a context which acknowledges the fact that differences in the route of administration could evoke differences in behavioral effect.

Four studies prior to this report have described tests using NMDA ligands and FCN procedures. Earlier investigators had worked with only two compounds, and both were noncompetitive antagonists. PCP and (+) *N*-allyl normetazocine (NANM) were each tested using a single-component FCN schedule (Bronson and Moerschbaecher, 1987; Snodgrass et al., 1997) and using multiple FCN schedule, the components of which included discriminative stimuli (cued) or excluded discriminative stimuli (uncued) (Doty et al., 1992; Picker et al., 1987; Snodgrass et al., 1997). Several observations from previous studies have interpretive relevance to this work. The analysis of PCP and NANM, using a multiple-component FCN schedule (FCN:FCN-SD), revealed unequal drug effects across signaled and unsignaled components (Picker et al., 1987). While large deficits in accuracy were observed in the FCN component, no changes in either rate or accuracy were observed in the signaled components (FCN-SD). In fact, this report indicated that 98% of all lever switches was reinforced during signaled components even at high doses of PCP (e.g., 3 mg/kg dose). When a memory-sensitive discrimination task is selectively impaired during components lacking explicit exteroceptive stimulus and no change in discriminative accuracy is observed in sensory-guided components, frequently it is inferred that a treatment-induced memory impairment occurred (Knepper and Kurylo, 1988; Miller et al., 1976; Opello et al., 1993). The multiple-schedule FCN study conducted by Picker and colleagues also demonstrated that subjects pre-injected with PCP could attend to external cues, and that cues remained perceptible. Other reports have linked NMDA antagonists to states of disorientation and inattention, and it is therefore appropriate to consider these variables when interpreting behavioral tests (Dai and Carey, 1994; Pontecorvo et al., 1991; Wozniak et al., 1990).

Noncompetitive NMDA antagonists have pharmacologic activity at dopamine transport sites. Thus, the question arises as to whether the performance effects are being mediated exclusively by the NMDA receptor. Importantly, the potency order for FCN disruption (MK-801>PCP>memantine) matches the potency order for NMDA receptor blockade, and does not match the potency order for binding to dopamine transport sites. Also, if indirect dopamine agonist actions were responsible for performance deficits, then ED50 values for PCP effects on accuracy would be different when co-administering a dopamine antagonist. We found them to be similar (Table 2). A final observation, which importantly supports NMDA transmission as the principal determinant of behavioral effects, is the selective reversal of accuracy deficits when SDZ EAA 494 was co-administered with NMDA. The performance profile, under conditions of competitive NMDA receptor blockade, is drastically changed by the addition of a selective agonist (see Table 2). Thus, the agonist/antagonist co-administration results strengthen the pharmacologic specificity of behavioral changes observed in these tests.

In summary, the present experiments show NMDA antagonist-induced disruption of a complex discrimination task. The experimental results, when taken together, offer support for NMDA receptor involvement in recalling learned discriminations. These findings are consistent with those obtained in other studies involving NMDA receptor function in declarative memory tasks (Baron and Moerschbaecher, 1996; Cohn and Cory-Slechta, 1993; France et al., 1991; Pontecorvo et al., 1991; Tan et al., 1989; Tonkiss and Rawlins, 1988). Furthermore, these results are consistent with a large clinical literature indicating amnestic symptoms upon human exposure to these compounds (Ghoneim et al., 1985; Javitt and Zukin, 1991; Krystal et al., 1994).

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