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Discriminative Stimulus Effects of Magnesium Chloride: Substitution Studies with Monoamine Uptake Inhibitors and N-Methyl-D-Aspartate Antagonists[1]

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ABSTRACT

Previous studies suggest that magnesium chloride may have discriminative stimulus effects that partially overlap with those of noncompetitive N-methyl-D-aspartate antagonists as well as certain monoamine uptake inhibitors. In our study, rats were trained to discriminate 100 mg/kg magnesium chloride from saline and its discriminative stimulus effects were characterized with respect to N-methyl-D-aspartate receptor and monoamine transporter functions in substitution tests. The discriminative stimulus effects of magnesium chloride were acquired within a moderate number of training sessions and showed dose-related substitution after either subcutaneous (3–300 mg/kg) or intracerebroventricular (0.3–300 μg) administration. The intracerebroventricular administration of magnesium chloride was over 4000 times more potent than its s.c. administration. The monoamine uptake inhibitors cocaine, GBR 12909, talsupram and citalopram fully substituted (≥90% magnesium-appropriate responses) for magnesium chloride in the majority of subjects tested and the group averages reached a maximum of 72 to 82% responses on the magnesium-appropriate lever. Based on relative potency analysis, the rank order of potency of these four drugs for producing magnesium-appropriate responses was talsupram = cocaine > citalopram = GBR 12909. The N-methyl-D-aspartate receptor antagonists dizocilpine, phencyclidine and NPC 17742 engendered maximum group averages of 49 to 65% responses on the magnesium-appropriate lever. The results suggest that the centrally mediated discriminative stimulus effects of magnesium chloride may be more directly related to interactions with monoamine neurotransmitter functions than to N-methyl-D-aspartate receptor blockade.

Mg^{++} is a mineral nutrient that is abundant in the brain as well as in the periphery (Aikawa, 1971). Several pharmacological studies have shown that systemically administered $MgCl_2$ has a profile of behavioral effects that are similar to those of indirect and direct monoamine receptor agonists. These include a heightening of aggression and an enhancement of cocaine-induced aggression, apomorphine-induced sniffing and amphetamine-induced locomotion in mice (Izenwasser *et al.*, 1986; Kantak, 1989; Kantak and Adlerstein, 1990). In a mouse conditioned place preference procedure, $MgCl_2$ was shown to induce a place preference (Lawley and Kantak, 1990b). Furthermore, $MgCl_2$ and amphetamine, but not haloperidol or pentobarbital, increased the magnitude of place preference induced by cocaine (Lawley and Kantak, 1990a). In groups of rats self-administering cocaine under FR 1 or FR 5 schedules of drug delivery, $MgCl_2$ was found to dose-dependently substitute for cocaine and to maintain a constant level of drug intake over a 10-day period (Kantak *et al.*, 1991). $MgCl_2$ also maintained responding above saline rates in substitution tests conducted under progressive-ratio schedules of drug delivery in cocaine-trained rats (Kantak *et al.*, 1991). Progressive-ratio breakpoints, however, were greater after cocaine availability than they were after $MgCl_2$ availability.

$MgCl_2$ can engender stimulus effects that share some common features with cocaine and NMDA associated ion channel blockers. Drug discrimination studies demonstrated that under a 2 mg/kg cocaine training dose condition, $MgCl_2$, as well as dizocilpine and PCP, engendered full substitution (≥90% responses) for cocaine in the majority of subjects tested (Kantak *et al.*, 1995). These same drugs failed to substitute for cocaine when subjects were trained to discriminate a higher dose (10 mg/kg) of cocaine. A competitive NMDA antagonist did not substitute for cocaine under either training dose condition. Others also have reported that the NMDA-associ-

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ABBREVIATIONS: ANOVA, analysis of variance; CSF, cerebrospinal fluid; DA, dopamine; FR, fixed-ratio; GBR 12909, 1-{2-[bis(4-fluorophenyl)-methoxy]ethyl}-4-(3-phenylpropyl)piperazine; 5-HT, 5-hydroxytryptamine; i.c.v., intracerebroventricular; Mg^{++}, magnesium; $MgCl_2$, magnesium chloride; NE, norepinephrine; NMDA, N-methyl-D-aspartate; NPC 17742, [2R,4R,5S-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid)]; PCP, phencyclidine.

ated channel blockers dizocilpine, PCP and ketamine partially substituted in cocaine-trained rats with a rank order corresponding to their potency for blocking the NMDA-associated ion channel (Koek *et al.*, 1989).

These findings raise the question of whether $MgCl_2$ itself would have discriminative stimulus effects independent of a prior drug history. Previous work, for example, has shown that rats will not self-administer $MgCl_2$ if they had no prior drug experience (Kantak *et al.*, 1990). However, conditioned place preference studies demonstrated that $MgCl_2$ could induce a change in preference in drug-naive mice (Lawley and Kantak, 1990b). The overlap in the behavioral effects of $MgCl_2$ with those of cocaine and NMDA antagonists described above prompted us to characterize the discriminative stimulus effects of $MgCl_2$ with respect to NMDA receptor and monoamine transporter functions. Neurochemical studies indicate that Mg^{++} functions not only as a noncompetitive NMDA antagonist by blocking the NMDA-associated ion channel (Wong and Kemp, 1991), but also as an important cofactor for monamine neurotransmitter binding to transporters (Amejdki-Chab *et al.*, 1992; Hendley *et al.*, 1988; White, 1975) and receptors (Hamblin and Creese, 1982; Lefkowitz *et al.*, 1976; Norman *et al.*, 1985; Salama *et al.*, 1982). Substitution tests were therefore conducted with the NMDA-associated ion channel blockers dizocilpine and PCP (Wong and Kemp,1991), the competitive NMDA antagonist NPC 17742 (Ferkany *et al.*, 1993), the nonselective monoamine uptake inhibitor cocaine (Koe, 1976), the selective DA uptake inhibitor GBR 12909 (van der Zee *et al.*, 1980), the selective NE uptake inhibitor talsupram and the selective 5-HT uptake inhibitor citalopram (Hyttel, 1982).

Methods

Subjects. Between experimental sessions, male Wistar rats (Charles River Breeding Labs, Portage, MI) were housed in individual stainless steel cages (24 × 18 × 18 cm). Home cages were located within a temperature- (74 ± 4° F) and a light- (0800 hr on, 2000 hr off) controlled vivarium. All rats were experimentally naive at the beginning of the study. The rats had continuous access to water in their home cages. Food (Agway Prolab Rodent Chow, Syracuse, NY) was restricted to 16 g/day and was provided after daily sessions. This ration of food maintained body weights at approximately 85% of *ad libitum* values. For the study duration, body weights ranged from 275 ± 12 to 435 ± 9 g in experiments involving substitution with various monoamine uptake inhibitors and NMDA antagonists and from 327 ± 5 to 478 ± 10 g in experiments involving peripheral vs. central administration of $MgCl_2$.

Apparatus. Each of four identical experimental chambers for rats (Gerbrands, model A, Waltham, MA) was equipped with two response levers that were mounted 7.6 cm apart. A pellet dispenser, which emitted an audible click when operated, delivered 45 mg food pellets (Noyes, Traditional Formula, Lancaster, NH) to a receptacle located between the levers. A sound-attenuating cubicle enclosed each chamber. In each cubicle, an overhead light provided general illumination and a fan provided ventilation and masked extraneous sounds. Experimental events were controlled by a 286 AT-compatible computer that was programmed in Medstate Notation and connected to an interface (Med Associates, East Fairfield, VT).

Drugs. The drugs studied were: cocaine hydrochloride and PCP hydrochloride (NIDA, Rockville, MD), dizocilpine maleate [(+)-MK 801] (Merck, Sharp and Dohme, West Point, PA), NPC 17742 (NOVA Pharmaceutical Corp., Baltimore, MD), $MgCl_2 \cdot 6H_2O$ (Fisher Scientific, Medford, MA), GBR 12909 dihydrochloride (Research Biochemicals Inc., Natick, MA), citalopram hydrobromide and talsupram hydrochloride (Lundbeck A/S, Copenhagen, Denmark). Except as noted below, drugs were dissolved and diluted to desired concentrations in either sterile 0.9% saline or sterile distilled water. Doses are expressed as salts (the anhydrous salt for $MgCl_2$). GBR 12909 (30 mg/ml) was dissolved in 0.9% saline and 1 M acetic acid (8:2), heated to 55°C and diluted to desired concentrations with 0.9% saline. NPC 17742 (30 mg/ml) was dissolved in 100 mM sodium hydroxide and diluted to desired concentrations with distilled water. $MgCl_2$ was either administered s.c. in a volume of 3.0 ml/kg body weight 15 min before the session (Kantak *et al.*, 1992, 1995) or infused i.c.v. in a volume of 5 μl over a 5-min period immediately before the session (Buck *et al.*, 1979; Willetts and Balster, 1988). Rats were gently held by the experimenter during infusions. The infusion cap was left in place for one min after the infusion. Other drugs were administered i.p. 15 min before the session in a volume of 1.0 ml/kg body weight.

Drug discrimination procedure. A total of 14 rats was trained to discriminate s.c. injections of 100 mg/kg $MgCl_2$ from saline. After administration of $MgCl_2$, 10 responses (FR 10) on one lever resulted in delivery of a food pellet, whereas after administration of saline, 10 responses on the other lever produced a food pellet. Training sessions were conducted daily (Monday to Friday) and lasted for a total of 15 min. Different sequences of randomly assigned saline and drug training sessions were used for each subject to ensure that any olfactory cues associated with the two levers would not bias the discrimination (Extance and Goudie, 1981). The training criteria consisted of at least 10 consecutive sessions in which ≥90% of responses were made on the injection-appropriate lever and the total number of responses on both levers did not exceed 12 before the first food pellet was delivered.

Drug testing procedure. Drug test sessions were conducted once or twice per week, with training sessions scheduled on intervening days. Test sessions were conducted only if performance during the preceding training session met the criteria previously described. Test sessions were identical to training sessions except that 10 responses on either lever resulted in delivery of a food pellet. In experiments involving substitution tests with monoamine uptake inhibitors and NMDA antagonists, a range of doses of s.c. $MgCl_2$ initially was examined in 10 subjects. Next, a range of doses of each of the four monoamine uptake inhibitors and each of the three NMDA antagonists was examined in subsets of six subjects to determine the degree to which each compound substituted for the 100 mg/kg training dose of $MgCl_2$. Most subjects received three or four of the seven test compounds; two subjects received six of the test compounds and one subject received all seven test compounds. Among the test compounds examined in each subject, at least one was an uptake inhibitor and one was a NMDA antagonist. Drugs were studied in different orders with different subjects, and saline test sessions were conducted on two or three occasions in each subject at various times during the experiment. In experiments involving peripheral *vs*. central administration of $MgCl_2$, the remaining subjects were implanted with a chronic indwelling 23-ga. stainless steel guide cannula ending 1 mm above the right lateral ventricle of the brain. Before surgery, rats were anesthetized with a combination of ketamine and xylazine (90 and 10 mg/kg, i.p., respectively). Coordinates for cannula placement were according to the atlas of Pellegrino *et al.* (1979): 0.6 mm posterior to bregma, 2.0 mm lateral from midline and 2.7 mm ventral from the surface of the skull. A 30-ga. stainless steel stylus was inserted into the guide cannula between infusions. The rats recovered for 4 days and then training with s.c. injections resumed. A range of doses of s.c. $MgCl_2$ and saline was subsequently examined in all four subjects. Next, a range of doses of i.c.v. $MgCl_2$ and saline was examined on two or three occasions in each subject to determine the degree to which i.c.v. $MgCl_2$ substituted for the 100 mg/kg training dose of $MgCl_2$.

Analysis of drug effects. The percentage of $MgCl_2$-appropriate responses was determined for each subject by dividing the number of $MgCl_2$-associated lever responses by the total number of responses emitted on both levers. Data were not included in the analysis if less

than 10 responses were made during a test session. When multiple determinations were obtained for a particular dose in the same animal (see above), values were averaged together for individual subjects before group averages were calculated. Where appropriate, the dose of a drug estimated to engender 50% $MgCl_2$-appropriate responses (ED_{50} ± 95% CI) was calculated by linear regression analysis over the ascending linear portion of the log dose-response curve. Using Pharmacologic Calculation System software, the log dose-response curves were further analyzed with the parallel line assay of Finney (1964) to ascertain differences in potency relative to $MgCl_2$. In the first study, each mg/kg dose of the test compounds was first converted to μmol/kg to account for differences in their molecular weights. In the second study, the relative potency between s.c. and i.c.v. $MgCl_2$ was computed after converting mg/kg s.c. $MgCl_2$ to μg/rat. For purposes of this study, full substitution for $MgCl_2$ was defined as ≥90% $MgCl_2$-appropriate responses.

Rates of responding were calculated for each subject by dividing the total number of responses on both levers by the total session length. The response-rate data were analyzed by a single-factor ANOVA and by post-hoc Dunnett's tests to compare the response rates after different doses with the rates after vehicle injections.

Results

Discrimination acquisition and effects of s.c. administered $MgCl_2$. The 10 rats used in this study met the training criteria after an average of 35 ± 2 training sessions (range 19–40 sessions). At criteria, rats made an average of 99% ± 0.3 responses on the $MgCl_2$-appropriate lever after injections of 100 mg/kg $MgCl_2$ and 98% ± 0.6 responses on the saline-appropriate lever after injections of saline. Throughout training, the rate of responding was slightly lower after $MgCl_2$ injections (.84 ± .01 responses per second) than after saline injections (1.1 ± .01 responses per second).

In substitution tests, $MgCl_2$ (3–300 mg/kg) engendered dose-related increases in the percentage of $MgCl_2$-appropriate responses. There was full substitution (≥90% $MgCl_2$-appropriate responses) in all subjects after administration of doses that were equal to or greater than the training dose (fig. 1, top panel). Regression analysis over the linear portion of the $MgCl_2$ dose-response curve revealed an ED_{50} = 20.4 mg/kg (95% CI = 6.3–66.1). Doses of $MgCl_2$ up to 30 mg/kg had little or no effect on the response rate compared to the saline control (fig. 1, bottom panel). Consistent with the training data, doses of $MgCl_2$ that were ≥100 mg/kg reduced the response rate significantly ($P < .05$).

Substitution tests with monoamine uptake inhibitors. Dose-related increases in the percentage of $MgCl_2$-appropriate responses (fig. 2, top panel) were engendered by 0.3 to 10.0 mg/kg cocaine, 3.0 to 17.8 mg/kg GBR 12909, 0.1 to 5.6 mg/kg talsupram and 1.0 to 10 mg/kg citalopram. Full substitution for $MgCl_2$ was observed in all six subjects after one or more doses of cocaine in the range of 1.0 to 10.0 mg/kg. For the group of six rats, the average percentage of $MgCl_2$-appropriate responses reached a maximum of 82% after 3.0 mg/kg cocaine and was 50% after 10.0 mg/kg cocaine. One or more doses of GBR 12909 engendered full substitution for $MgCl_2$ in five of six subjects in the dose range of 3.0 to 17.8 mg/kg. Averaged for the group of rats, the maximum percentage of $MgCl_2$-appropriate responses reached 74% after 17.8 mg/kg GBR 12909. One or more doses of talsupram also engendered full substitution for $MgCl_2$ in five of the six subjects in the dose range of 0.1 to 5.6 mg/kg. For the group of six rats, the maximum average percentage of $MgCl_2$-appropriate responses reached 72% after 5.6 mg/kg talsupram. Finally, full substitution for $MgCl_2$ was observed in five of the six subjects after citalopram in the dose range of 3.0 to 10.0 mg/kg. The maximum average percentage of $MgCl_2$-appropriate responses reached 82% after 10.0 mg/kg citalopram for the group of six rats. Based on ED_{50} and relative potency analysis (table 1), the rank order of potency of these drugs for producing $MgCl_2$-appropriate responses was talsupram = cocaine > citalopram = GBR 12909 > $MgCl_2$. Each monoamine uptake inhibitor, except cocaine, produced dose-related decreases in the response rate, with significant ($P < .05$) reductions in the rate after the highest doses tested (fig. 2, bottom panel).

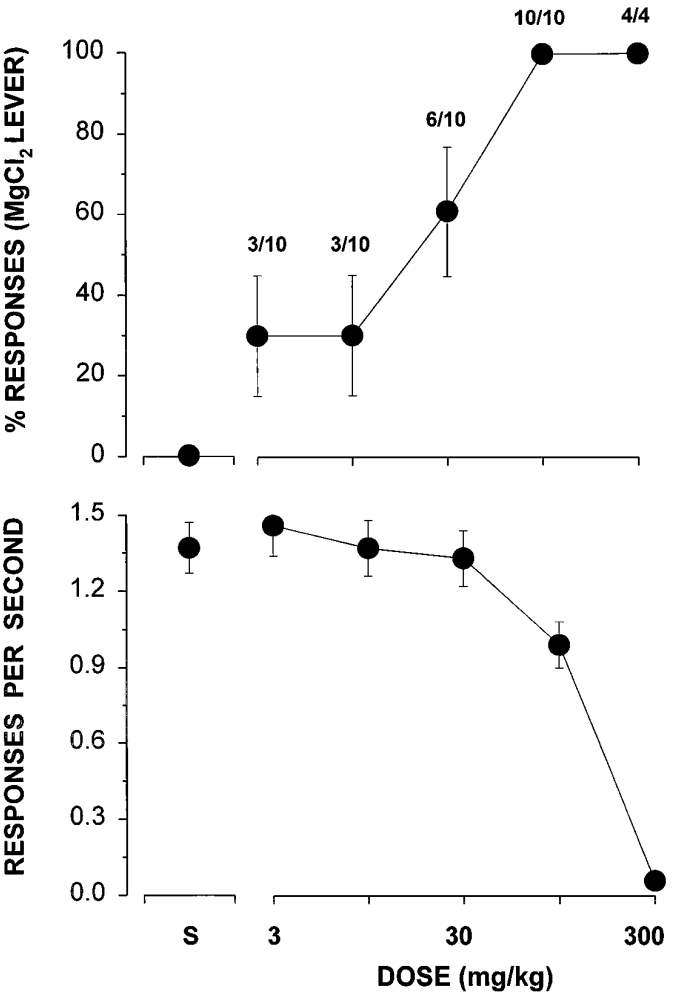

Fig. 1. Effects of $MgCl_2$ in rats trained to discriminate 100 mg/kg $MgCl_2$ from saline. Abscissae: dose, log scale; ordinates: percentage of responses on the $MgCl_2$-appropriate lever (top panel) and rate of responding (bottom panel). Points are the means (± S.E.M.) based on 10 rats, except as noted. The numbers above each point refer to the proportion of rats that made ≥90% $MgCl_2$-appropriate responses after administration of the dose indicated on the abscissa. Points above S show the effects of saline.

Substitution tests with NMDA antagonists. Administration of 0.03 to 0.18 mg/kg dizocilpine and 0.3 to 3.0 mg/kg PCP resulted in dose-related changes in the percentage of $MgCl_2$-appropriate responses (fig. 3, top). Full substitution for $MgCl_2$ was observed in three of six subjects after one or

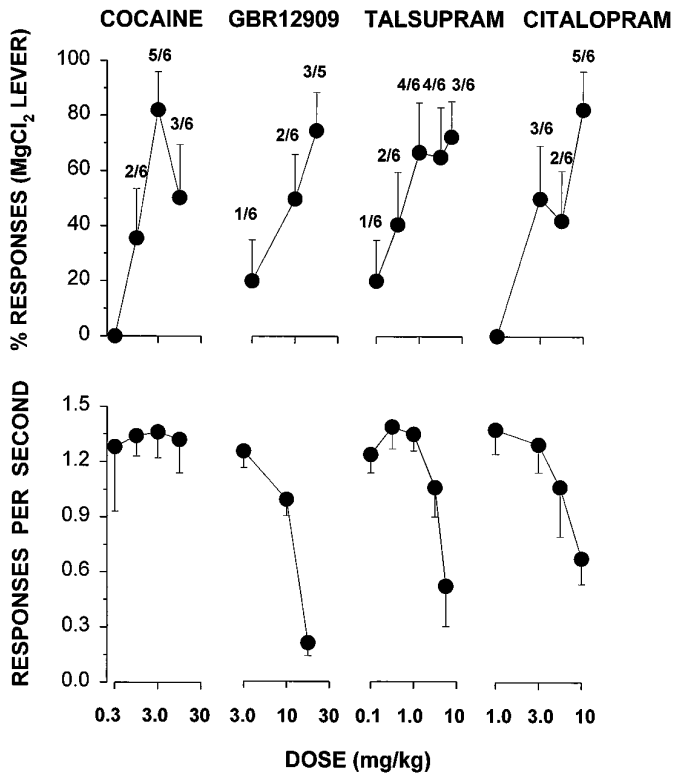

Fig. 2. Effects of cocaine, GBR 12909, talsupram and citalopram in rats trained to discriminate 100 mg/kg $MgCl_2$ from saline. Points are the means (± S.E.M.) based on six rats, except as noted. Saline values and other details are as in figure 1.

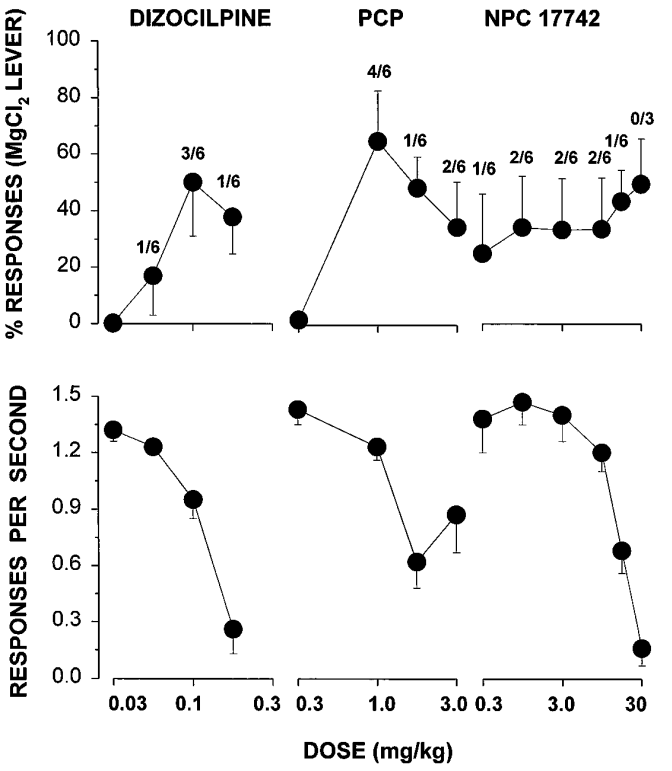

Fig. 3. Effects of dizocilpine, PCP and NPC 17742 in rats trained to discriminate 100 mg/kg $MgCl_2$ from saline. Points are the means (± S.E.M.) based on six rats, except as noted. Saline values and other details are as in figure 1.

more doses of dizocilpine in the range of 0.056 to 0.18 mg/kg. The maximum average percentage of $MgCl_2$-appropriate responses reached 50% after 0.1 mg/kg dizocilpine for the group of six rats. One or more doses of PCP in the range of 1.0 to 3.0 mg/kg engendered full substitution for $MgCl_2$ in four of six subjects and the maximum average percentage of $MgCl_2$-appropriate responses reached 65% after 1.0 mg/kg PCP for the group of six rats. The competitive NMDA antagonist NPC 17742 (0.3–30 mg/kg) did not produce any dose-related change in the percentage of $MgCl_2$-appropriate responses (fig. 3, top). Responses were mainly made on the saline-associated lever in most animals after each dose of NPC 17742. The average percentage of $MgCl_2$-appropriate responses was 49% or less across the range of doses for the group of rats. Although NPC 17742 produced full substitution for $MgCl_2$ in four of six subjects, its occurrence was irregularly related to dose (between 0.3–17.8 mg/kg for individual animals). As shown in the bottom of figure 3, each NMDA antagonist produced dose-related decreases in the response rate, with significant ($P < .05$) reductions in the rate after the two highest doses tested.

Effects of peripherally vs. centrally administered $MgCl_2$. In experiments involving s.c. *vs.* i.c.v. injections of $MgCl_2$, the rats met the training criteria after an average of 46 ± 4 training sessions (range 24–57 sessions). After training, s.c. injections of $MgCl_2$ (3–178 mg/kg) engendered dose-related increases in the percentage of $MgCl_2$-appropriate responses, with an ED_{50} = 31.6 mg/kg (95% CI = 15.1–66.1). Full substitution was observed in all subjects after doses that were equal to or greater than the training dose (fig. 4, top left). These results are quite similar to those reported in figure 1.

TABLE 1

ED_{50} and relative potency (95% CI range) for producing $MgCl_2$-appropriate responses after substitution tests with cocaine and selective monoamine uptake inhibitors and with s.c. $MgCl_2$ vs. i.c.v. $MgCl_2$

Drug	ED_{50} mg/kg	ED_{50} μmol/kg	Relative Potency	95% CI
$MgCl_2$	20.4	215	1.0	
Cocaine	1.4	4.1	0.014	0.005–0.030
Talsupram	0.7	2.0	0.008	0.001–0.025
Citalopram	4.6	11.4	0.048	0.019–0.125
GBR 12909	8.9	17.0	0.063	0.015–0.167

Route	ED_{50} mg/kg	ED_{50} μg/rat	Relative Potency	95% CI
s.c. $MgCl_2$	31.6	13,490	1.0	
i.c.v. $MgCl_2$		3.4	2.4×10^{-4}	0.9×10^{-4}–6×10^{-4}

Administration of i.c.v. $MgCl_2$ (0.3–300 μg) engendered dose-related, biphasic changes in the percentage of $MgCl_2$-appropriate responses (fig. 4, top right). Full substitution for the s.c. administration of 100 mg/kg $MgCl_2$ was observed in all subjects after 30 μg $MgCl_2$ and in three of the four subjects after 100 μg $MgCl_2$. The administration of 300 μg $MgCl_2$ resulted in much less $MgCl_2$-appropriate responses in all subjects. Based on ED_{50} and relative potency analyses over the linear ascending portion of the log dose-response curve, the i.c.v. administration of $MgCl_2$ was more than 4000 times more potent than the s.c. administration of $MgCl_2$ for producing $MgCl_2$-appropriate responses (table 1). The response rates were not significantly altered by any dose of i.c.v. ad-

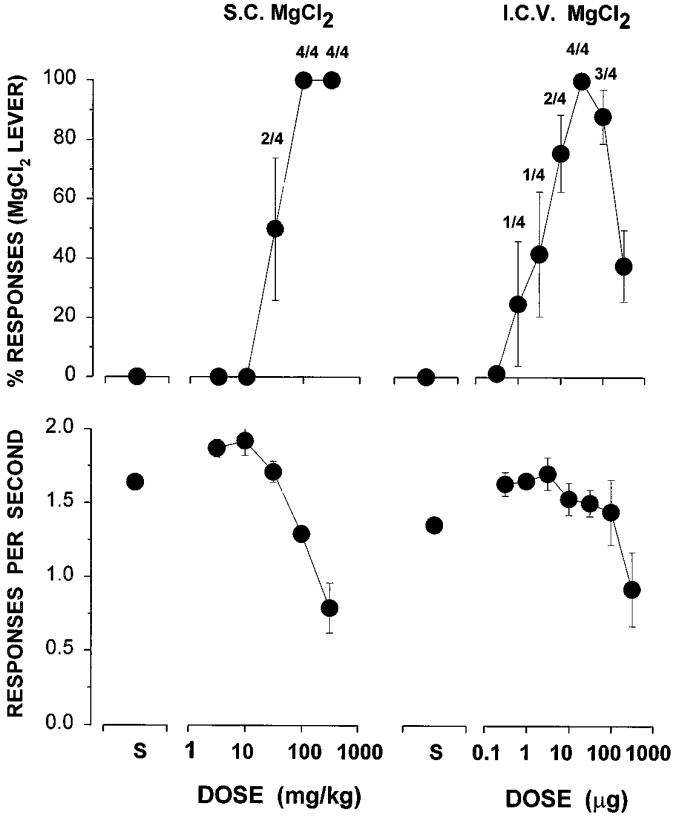

Fig. 4. Effects of s.c. $MgCl_2$ (left) and i.c.v. $MgCl_2$ (right) in rats trained to discriminate 100 mg/kg $MgCl_2$ from saline. Points are the means (± S.E.M.) based on four rats. Other details are as in figure 1.

ministered $MgCl_2$ (fig. 4, bottom right), although a rate-decreasing effect was evident in some rats after 300 μg. As were reported in figure 1, the response rates were significantly decreased ($P < .05$) after doses that were ≥ 100 mg/kg s.c. $MgCl_2$ (fig. 4, bottom left).

Discussion

Our results indicate that $MgCl_2$ has discriminative stimulus effects that were reproducibly acquired and involved central mechanisms. Furthermore, the discriminative stimulus effects of $MgCl_2$ were less similar to other drugs with NMDA antagonist effects and more similar to monoamine uptake inhibitors. After a moderate number of training sessions, 100 to 300 mg/kg $MgCl_2$ fully substituted for the 100 mg/kg training dose. Lower doses engendered fewer responses on the $MgCl_2$-appropriate lever. The S-shaped dose-response function that resulted from training rats to discriminate 100 mg/kg $MgCl_2$ is a general feature of most drugs having discriminative stimulus effects (see Jarbe, 1989 for review).

An important question that can be raised relates to the pharmacological specificity of the discriminative stimulus effects of $MgCl_2$. Because the concentration of the 100 mg/kg training dose was 350 mM, it is possible that the solution's hypertonicity rather than its pharmacological properties caused the cuing effects of $MgCl_2$. Controls for hypertonicity were not used as part of our experiments. Arguments against this possibility include the fact that the 30 μg solution was hypotonic (62.5 mM), yet its i.c.v. infusion fully substituted for the 100 mg/kg training dose. Other studies have shown that the i.v. delivery of hypertonic (335 mM) NaCl did not alter self-administration responding any differently than physiological (154 mM) NaCl (Kantak *et al.*, 1990; 1991). Furthermore, the differential effects of the NMDA-associated ion channel blockers *vs.* monoamine uptake inhibitors and the relatively weak effects of NPC 17742 in substitution tests suggest some degree of pharmacological specificity to the discriminative stimulus effects of $MgCl_2$ (Colpaert *et al.*, 1979; Terry *et al.*, 1994).

The biphasic, dose-related changes in drug-appropriate responses after i.c.v. administration of $MgCl_2$ have generally not been observed after the i.c.v. administration of other drugs (*e.g.,* Sannerud *et al.*, 1991; Willetts and Balster, 1988; Wood *et al.*, 1987). In these other studies, the dose-related changes in drug-appropriate responses tended to be linear. In our study, drug-appropriate responses were linearly related to $MgCl_2$ dose, up to 100 μg. The i.c.v. infusion of our highest dose of $MgCl_2$ (300 μg) resulted in much less $MgCl_2$-appropriate responses than lower doses (30 or 100 μg). The effects of 300 μg $MgCl_2$ were most likely nonspecific, which could be related to a severe depression of cortical neurons that is produced by high concentrations of centrally applied Mg^{++} ions (Kelly *et al.*, 1969).

In other drug discrimination studies, i.c.v. infusions of 80 μg cocaine (Wood *et al.*, 1987), 150 μg PCP (Willetts and Balster, 1988) and 44 μg midazolam (Sannerud *et al.*, 1991) fully substituted for their systemic injections, suggesting central mediation of their discriminative stimulus effects. Furthermore, the potency of these drugs was up to 40-fold greater after direct infusion into the lateral ventricle of the brain. From comparing s.c. and i.c.v. administration of $MgCl_2$, it is apparent that all doses of i.c.v. administered $MgCl_2$ studied would have engendered saline-appropriate responses if systemically administered. Therefore, diffusion out of the brain could not have accounted for the full substitution by 30 μg i.c.v. $MgCl_2$ for the 100 mg/kg s.c. training dose. The i.c.v. administration of 17 and 50 μg $MgCl_2$ has previously been shown to increase CSF concentrations of Mg^{++} by 2- and 6-fold, respectively, and to dose-dependently decrease audiogenic seizure severity in rats without altering serum concentrations of Mg^{++} (Buck *et al.*, 1979).

Although cannulae placements were not verified in this study, central mediation of the discriminative stimulus effects of $MgCl_2$ also is suggested by the finding that $MgCl_2$ was more than 4000 times more potent after its central *vs.* peripheral administration. The greater increase in potency after direct infusion of $MgCl_2$ into the lateral ventricle of the brain compared to other drugs (Sannerud *et al.*, 1991; Willetts and Balster, 1988; Wood *et al.*, 1987) may be related to the fact that only small amounts of $MgCl_2$ are absorbed and distributed in the brain after its peripheral administration (Hilmy and Somjen, 1968), yet central neurons are quite sensitive to a relatively small concentration (1 mM) of iontophoretically applied Mg^{++} (Somjen and Kato, 1968). This may account for the relatively large dose of $MgCl_2$ (100 mg/kg or an average of 44,000 μg/rat) necessary for maintaining discriminative stimulus control after a s.c. injection and the relatively small dose of $MgCl_2$ (30 μg) necessary for full substitution after infusion into the lateral ventricle of the brain.

Among its biochemical effects in the brain, Mg^{++} functions as a noncompetitive NMDA antagonist by binding to a spe-

cific Mg^{++} recognition site and blocking the NMDA-associated ion channel (Wong and Kemp, 1991). However, the results of our study suggest that the discriminative stimulus effects of $MgCl_2$ were distinct from other NMDA antagonists. The NMDA-associated ion channel blockers PCP and dizocilpine only partially substituted for $MgCl_2$. The $MgCl_2$-like effects of NPC 17742 were weak and not systematically related to dose. Responses were mostly made on the saline-associated lever over the entire dose range. A previous study in PCP-trained rats demonstrated that 80 mg/kg $MgCl_2$ engendered only 21% PCP-appropriate responses, yet the rate of responding was significantly decreased, suggesting that a behaviorally effective dose of $MgCl_2$ was used (Jortani *et al.*, 1992). In other studies, a competitive NMDA antagonist did not substitute for PCP (Willetts and Balster, 1988) and PCP did not substitute for a competitive NMDA antagonist (Willetts *et al.*, 1989). Taken together, these findings show that the discriminative stimulus effects of $MgCl_2$ do not completely overlap with those of other NMDA-associated ion channel blockers or competitive NMDA antagonists, which also do not completely overlap with each other.

The discriminative stimulus effects of $MgCl_2$ do appear to overlap with the nonselective monoamine uptake inhibitor cocaine and the selective monoamine uptake inhibitors GBR 12909, talsupram and citalopram. The nonselective monoamine uptake inhibitor cocaine engendered 82% $MgCl_2$-appropriate responses after a dose of 3 mg/kg. These results are consistent with the study showing that 30 to 300 mg/kg $MgCl_2$ engendered 73 to 96% cocaine-appropriate responses in rats trained to discriminate 2 mg/kg cocaine (Kantak *et al.*, 1995). The selective DA, NE and 5-HT uptake inhibitors fully substituted for $MgCl_2$ in most animals and engendered a higher degree of $MgCl_2$-appropriate responses than the NMDA antagonists. These findings suggest that the discriminative stimulus effects of $MgCl_2$ may involve more than its neurochemical effect at the NMDA receptor. Indeed, Mg^{++} has multiple biochemical sites of action in the brain (Ebel and Gunther, 1980). The discriminative stimulus effects of $MgCl_2$ could involve interactions of $MgCl_2$ at either monoamine uptake sites, by inhibiting monoamine transporter functions, or monoamine receptor sites, by increasing monoamine binding and receptor activation. Although low mM concentrations of Mg^{++} are necessary for maintaining normal rates of monoamine uptake (*e.g.,* Amejdki-Chab *et al.*, 1992; Hendley *et al.*, 1988; White, 1975), Mg^{++} concentrations of more than 10 mM can inhibit the uptake of DA and reduce specific binding of [^{3}H]GBR 12783 or [^{3}H]GBR 12935 with an IC_{50} value of 16 mM (Amejdki-Chab *et al.*, 1992; Janowsky *et al.*, 1986). From the i.c.v. infusion of 30 μg $MgCl_2$, which fully substituted for the 100 mg/kg training dose, the concentration of $MgCl_2$ delivered to the lateral ventricle was 62.5 mM. This concentration would be diluted at least 100-fold after the distribution of $MgCl_2$ into brain tissue. If DA, NE and 5-HT transporters are similarly inhibited by concentrations of Mg^{++} of more than 10 mM *in vivo*, then the concentration of $MgCl_2$, either from s.c. injections or i.c.v. infusions, may not have reached high enough levels to inhibit monoamine transporter functions in brain tissue.

Mg^{++} has been shown to be necessary for the binding of monoamine neurotransmitters at D_1, D_2, α_2-NE, β-NE and 5-HT_{1a} receptors (*e.g.,* Hamblin and Creese, 1982; Lefkowitz *et al.*, 1976; Norman *et al.*, 1985; Salama *et al.*, 1982). Dopamine binding studies, for example, have demonstrated that Mg^{++} increases the B_{max} associated with ^{3}H-spiroperidol binding in rat striata by 200% above control (Usdin *et al.*, 1980). This increase is seen with concentrations of Mg^{++} as low as 0.1 to 1.0 mM. Mg^{++} does not actually increase the number of receptors, but prevents the time-dependent degradation of ligand binding to the receptor. Other studies (De Vries and Beart, 1985; Hamblin and Creese, 1982) demonstrated that Mg^{++} enhances DA affinity at D_2 receptors by a factor of 1000 (a μM to nM potency change). Given that the monoamine uptake inhibitors used in our study could be regarded as indirect monoamine receptor agonists, it is more likely that monoamine receptor activation, rather than monoamine uptake inhibition, could be the common mechanism by which $MgCl_2$, cocaine, GBR 12909, talsupram and citalopram engendered a high degree of $MgCl_2$-appropriate responses. Further characterization of the discriminative stimulus effects of $MgCl_2$ should include antagonism studies with NMDA and selective monoamine receptor blockers to evaluate the role of the Mg^{++} site on the NMDA receptor complex *vs*. monoamine receptor activation in mediating the effects of $MgCl_2$. Furthermore, NE mechanisms may be particularly important for the discriminative stimulus effects of $MgCl_2$. The potency of talsupram was equal to that of cocaine and more than that of citalopram and GBR 12909 in engendering $MgCl_2$-appropriate responses. Interestingly, as with $MgCl_2$ (Kantak *et al.*, 1995), selective NE uptake inhibitors engendered cocaine-appropriate responses under a low-dose, but not a high-dose, training condition (Spealman, 1995; Terry *et al.*, 1994). To eliminate any pharmacokinetic differences among talsupram, cocaine, citalopram and GBR 12909, additional substitution studies with the i.c.v. administration of these uptake inhibitors should also be conducted to determine if talsupram is still the most potent in engendering $MgCl_2$-appropriate responses. If the rank order of potency is the same in this context as it was when these drugs were given systemically, then these potency differences would indicate a predominance of NE mechanisms in discriminative stimulus effects of $MgCl_2$.

In summary, the discriminative stimulus effects of $MgCl_2$ are novel findings and add to the growing evidence that $MgCl_2$ can have pharmacological effects (*e.g.,* Buck *et al.*, 1979; Kantak *et al.*, 1992; 1995; McIntosh *et al.*, 1989; Smith *et al.*, 1993). The discriminative stimulus effects of $MgCl_2$ appear to be centrally mediated and may involve interactions of $MgCl_2$ at monoamine receptor sites.

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References

Aikawa, J. K.: The biochemical and cellular functions of magnesium. *In* First International Symposium on Magnesium Deficit in Human Pathology, ed. by J. Durlach, pp. 39–53, Villel, France, 1971.

Amejdki-Chab, N., Benmansour, S., Costentin, J. and Bonnet, J.-J.: Effects of several cations on the neuronal uptake of dopamine and the specific binding of [^{3}H]GBR 12783: Attempts to characterize the Na^+ dependence of the neuronal transport of dopamine. J. Neurochem. **59:** 1795–1804, 1992.

Buck, D. R., Mahoney, A. W. and Hendricks, D. G.: Effects of cerebral intraventricular magnesium injections and a low magnesium diet on nonspecific excitability level, audiogenic seizure susceptibility and serotonin. Pharmacol. Biochem. Behav. **10:** 487–491, 1979.

Colpaert, F. C., Niemegeers, C. J. E. and Janssen, P. A. J.: Discriminative stimulus properties of cocaine: Neuropharmacological characteristics as de-

rived from stimulus generalization experiments. Pharmacol. Biochem. Behav. **10:** 535–546, 1979.

De Vries, D. J. and Beart, P. M.: Magnesium ions reveal nanomolar potency of dopamine at [^{3}H]spiperone labelled D-2 receptors in rat corpus striatum. Eur. J. Pharmacol. **109:** 417–419, 1985.

Ebel, H. and Gunther, T.: Magnesium metabolism: A review. J. Clin. Chem. Clin. Biochem. **8:** 257–270, 1980.

Extance, K. and Goudie, A. J.: Inter-animal olfactory cues in operant drug discrimination procedures in rats. Psychopharmacology **73:** 363–371, 1981.

Ferkany, J. W., Hamilton, G. S., Patch, R. J., Huang, Z., Borosky, S. A., Bednar, D. L., Jones, B. E., Zubrowski, R., Willets, J. and Karbon, E. W.: Pharmacological profile of NPC 17742 [2R,4R,5S-(2-amino-4,5- (1,2-cyclohexyl)-7-phosphonoheptanoic acid)], a potent, selective and competitive N-methyl-D-aspartate receptor antagonist. J. Pharmacol. Exp. Ther. **264:** 256–264, 1993.

Finney, D. J.: Statistical Methods in Biological Assay, 2nd ed., Hafner, New York, 1964.

Hamblin, M. W. and Creese, I.: ^{3}H-Dopamine binding to rat striatal D-2 and D-3 sites: Enhancement by magnesium and inhibition by guanine nucleotides and sodium. Life Sci. **30:** 1587–1595, 1982.

Hendley, E. D., Whittemore, S. R., Chaffee, J. E. and Ehrlick, Y. H.: Regulation of norepinephrine uptake by adenine nucleotides and divalent cations: Role for extracellular protein phosphorylation. J. Neurochem. **50:** 263–273, 1988.

Hilmy, M. I. and Somjen, G. G.: Distribution and tissue uptake of magnesium related to its pharmacological effects. Am. J. Physiol. **214:** 406–413, 1968.

Hyttel, J.: Citalopram–Pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. Prog. Neuropsychopharmacol. Biol. Psychiatry **6:** 277–295, 1982.

Izenwasser, S. E., Garcia-Valdez, K. and Kantak, K. M.: Stimulant-like effects of magnesium on aggression in mice. Pharmacol. Biochem. Behav. **25:** 1195–1199, 1986.

Janowsky, A., Berger, P., Vocci, F., Labarca, R., Skolnick, P. and Paul, S. M.: Characterization of sodium-dependent [^{3}H]GBR-12935 binding in brain: A radioligand for selective labelling of the dopamine transport complex. J. Neurochem. **46:** 1272–1276, 1986.

Jarbe, T. U. C.: Discrimination learning with drug stimuli: Methods and applications. *In* Neuromethods: Psychopharmacology, vol. 13, ed. by A. A. Boulton, G. B. Baker and A. J. Greenshaw, pp. 513–563, Humana Press, Clifton, NJ, 1989.

Jortani, S. A., Willetts, J. and Balster, R. L.: Systemic magnesium chloride administration fails to produce phencyclidine-like discriminative stimulus effects in rats. Neurosci. Lett. **135:** 136–138, 1992.

Kantak, K. M., Bourg, J. F. and Lawley, S. I.: Failure of magnesium to maintain self-administration in cocaine-naive rats. Pharmacol. Biochem. Behav. **36:** 9–12, 1990.

Kantak, K. M., Edwards, M. A. and Spealman, R. D.: Effects of N-methyl-D-aspartate antagonists in rats discriminating different doses of cocaine: comparison with direct and indirect dopamine agonists. J. Pharmacol. Exp. Ther. **274:** 657–665, 1995.

Kantak, K. M., Lawley, S. I., Wasserman, S. J. and Bourg, J. F.: Magnesium-maintained self-administration responding in cocaine-trained rats. Psychopharmacology **104:** 527–535, 1991.

Kantak, K. M. and Adlerstein, L. K.: Enhancement of apomorphine and l-amphetamine induced behaviors by magnesium. Pharmacol. Biochem. Behav. **36:** 29–33, 1990.

Kantak, K. M., Wasserman, S. J., Lawley, S. I. and O'Connor, T.: Acute and multiple injection effects of $MgCl_2$ on responding maintained by cocaine, extinction from cocaine, glucose + saccharin, and food. Pharmacol. Biochem. Behav. **41:** 415–423, 1992.

Kantak, K. M.: Magnesium alters the potency of cocaine and haloperidol on mouse aggression. Psychopharmacology **99:** 181–188, 1989.

Kelly, J. S., Krnjevic, K. and Somjen, G. G.: Divalent cations and electrical properties of cortical cells. J. Neurobiol. **2:** 197–208, 1969.

Koek, W., Colpaert, F. C., Woods, J. H. and Kamenka, J.-M.: The phencyclidine (PCP) analog N-[1-(2-benzo(B)thiophenyl)cyclohexyl] piperidine shares cocaine-like but not other characteristic behavioral effects with PCP, ketamine and MK-801. J. Pharmacol. Exp. Ther. **250:** 1019–1027, 1989.

Koe, B. K.: Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J. Pharmacol. Exp. Ther. **199:** 649–661, 1976.

Lawley, S. I. and Kantak, K. M.: Post-conditioning effects of magnesium on cocaine induced place preference in mice. Pharmacol. Biochem. Behav. **36:** 531–538, 1990a.

Lawley, S. I. and Kantak, K. M.: Magnesium induced place preference in mice. Pharmacol. Biochem. Behav. **36:** 539–545, 1990b.

Lefkowitz, R. J., Mullikin, D. and Caron, M. G.: Regulation of β-adrenergic receptors by guanyl-5′-yl imidodiphosphate and other purine nucleotides. J. Biol. Chem. **15:** 4686–4692, 1976.

McIntosh, T. K., Vink, R., Yamakami, I. and Faden, A. I.: Magnesium protects against neurological deficit after brain injury. Brain Res. **482:** 252–260, 1989.

Norman, A. B., Battaglia, G. and Creese, I.: [^{3}H]WB4101 labels the 5-HT_{1a} serotonin receptor subtype in rat brain: Guanine nucleotide and divalent cation sensitivity. Mol. Pharmacol. **28:** 487–494, 1985.

Pellegrino, L. J., Pellegrino, A. S. and Cushman, A. J.: A Stereotaxic Atlas of the Rat Brain, 2nd ed., Plenum Press, New York, 1979.

Salama, A. I., Lin, L. L., Repp, L. D. and U'Prichard, D. C.: Magnesium reduces affinities of antagonists at rat cortex α_2-adrenergic receptors labelled with ^{3}H-clonidine: Evidence for heterogeneity of α_2-receptor conformations with respect to antagonists. Life Sci. **30:** 1305–1311, 1982.

Sannerud, C. A., Ator, N. A. and Griffiths, R. R.: Comparison of the discriminative stimulus effects of midazolam after intracranial and peripheral administration in the rat. Life Sci. **49:** 261–268, 1991.

Smith, D. H., Okiyama, K., Gennarelli, T. A. and McIntosh, T. K.: Magnesium and ketamine attenuate cognitive dysfunction following experimental brain injury. Neurosci. Lett. **157:** 211–214, 1993.

Somjen, G. G. and Kato, G.: Effects of magnesium and calcium on neurones in the central nervous system. Brain Res. **9:** 161–164, 1968.

Spealman, R. D.: Noradrenergic involvement in the discriminative stimulus effects of cocaine in squirrel monkeys. J. Pharmacol. Exp. Ther. **275:** 53–62, 1995.

Terry, P., Witkin, J. M. and Katz, J. L.: Pharmacological characterization of the novel discriminative stimulus effects of a low dose of cocaine. J. Pharmacol. Exp. Ther. **270:** 1041–1048, 1994.

Usdin, T. B., Creese, I. and Snyder, S. H.: Regulation by cations of [^{3}H]spiroperidol binding associated with dopamine receptors of rat brain. J. Neurochem. **34:** 669–676, 1980.

van der Zee, P., Koger, H. S., Gootjes, J. and Hespe, W.: Aryl 1,4-dialk(en)yl-piperazines as selective and very potent inhibitors of dopamine uptake. Eur. J. Med. Chem. **15:** 363–370, 1980.

White, T. D.: A role of divalent cations in the uptake of noradrenaline by synaptosomes. J. Neurochem. **24:** 1037–1042, 1975.

Willetts, J., Bobelis, D. J. and Balster, R. L.: Drug discrimination based on the competitive N-methyl-D-aspartate antagonist NPC 12626. Psychopharmacology **99:** 458–462, 1989.

Willetts, J. and Balster, R. L.: The discriminative stimulus effects of N-methyl-D-aspartate antagonists in phencyclidine-trained rats. Neuropharmacology **27:** 1249–1256, 1988.

Wong, E. H. F. and Kemp, J. A.: Sites for antagonism of the N-methyl-D-aspartate receptor channel complex. Annu. Rev. Pharmacol. Toxicol. **31:** 401–425, 1991.

Wood, D. M., Retz, K. R. and Emmett-Oglesby, M. W.: Evidence of a central mechanism mediating tolerance to the discriminative stimulus properties of cocaine. Pharmacol. Biochem. Behav. **28:** 401–406, 1987.

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