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Sensitive and rapid behavioral differentiation of *N*-methyl-D-aspartate receptor antagonists

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Abstract. Behavioral effects of PCP-type noncompetitive antagonists of *N*-methyl-D-aspartate (NMDA) receptors overlap with those of a host of other centrally acting compounds. In the present experiment, locomotor activity and performance on an inverted screen test in untrained mice were used to differentiate PCP-type noncompetitive NMDA antagonists from other drug classes. These uncompetitive NMDA antagonists [PCP, dizocilpine, (−)-MK-801, TCP, (+)-SKF 10,047, dextrorphan, ketamine] produced dose-related increases in locomotor activity and the percentage of mice falling off an inverted, elevated wire mesh screen. Both effects demonstrated stereoselectivity, occurred at comparable dose levels, and were within the range of doses producing other biological effects (e.g., anticonvulsant). The potencies of these drugs for producing behavioral effects were positively correlated with affinities for PCP ([^{3}H]MK-801) but not σ([^{3}H]SKF 10,047) receptors. Although muscarinic antagonists (benactyzine, atropine) produced effects in the same direction, locomotor stimulation was small and occurred at lower doses than those inducing screen failures. Competitive NMDA antagonists (LY 274614, LY 233536, CPP, NPC 12626), σ receptor ligands (DTG, dextromethorphan), postsynaptic dopamine agonists (quinpirole, SKF 38393) and antagonists (haloperidol, SCH 39166), and some depressant compounds (morphine, diazepam) increased failures on the screen test but decreased locomotor activity. Ligands of the polyamine regulatory site of the NMDA receptor (ifenprodil, SL 82.0715–10) and the AMPA receptor antagonist NBQX decreased locomotor activity without increasing screen failures. An antagonist of the strychnine-insensitive glycine receptor (7-chlorokynurenic acid) did not affect performance on either test. Psychomotor stimulants (cocaine and methamphetamine) stimulated locomotor activity without affecting screen performance. The only false positives occurred with barbiturates (pentobarbital, phenobarbital). Nonetheless, the present procedure demonstrates excellent sensitivity and power for rapid discrimination of uncompetitive NMDA antagonists.

Key words: NMDA receptor antagonists – Dissociative anesthetics – Behavioral effects – Locomotor activity – Mice

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The dissociative anesthetic phencyclidine (PCP) is an abused substance (Balster 1987; National Institute on Drug Abuse 1990) which produces psychotomimetic effects in humans characterized by delirium, excitation, and marked affective changes (Burns and Lerner 1981; Domino and Luby 1981). In rodents, PCP produces ataxia, head weaving, hyperexcitability, and increases in locomotion (cf. Wilmot et al. 1989; Willetts et al. 1990). Although PCP has prominent effects on monoaminergic neural systems (cf. Chen et al. 1959; Greenberg and Segal 1985) and also binds to σ receptors (cf. Wong et al. 1988), a substantial body of evidence suggests that noncompetitive interactions of PCP with *N*-methyl-D-aspartate (NMDA) receptors play a major role in its pharmacological actions (cf. Lodge and Anis 1982; Lodge and Johnson 1990) and in the induction of its behavioral effects (cf. Balster 1987; Balster and Willetts 1988). The NMDA receptor, a glutamate receptor subtype, is a ligand-gated receptor complex with multiple regulatory sites. NMDA receptors have been implicated in the regulation of a host of biochemical and behavioral events of significance including learning and memory, anxiety, depression, parkinsonism, epilepsies, and neurodegeneration (Meldrum 1985; Choi 1988; Olney 1989; Carlsson and Carlsson 1990; Trullas and Skolnick 1990). Not surprisingly, the NMDA receptor has been the focus of a range of drug discovery efforts in the quest for pharmacological therapies for these central nervous system (CNS) disorders. Elimination or reduction in the psychotomimetic effects which characterize PCP and related NMDA antagonists has been one guiding strategy for drug development in this area.

Convergent data from electrophysiological, biochemical, and behavioral experiments point to the ion channel of the NMDA receptor as a target for induction of the confluence of behavioral and subjective effects characteristic of PCP. Thus, like PCP, other drugs which bind noncompetitively to the NMDA receptor ion channel also produce behavioral effects similar to those of PCP (Clineschmidt et al. 1982; Balster and Willetts 1988; Tricklebank et al. 1989; Székely et al. 1991). Competitive inhibitors of the NMDA receptor (i.e., those that bind to the glutamate site) have also been reported to produce PCP-like behavioral effects. Competitive blockers result in ataxia, stereotypies, and increased locomotion (Boast and Pastor 1987; Tricklebank et al. 1989; Koek and Colpaert 1990; Liljequist 1991), produce PCP-like catalepsy in pigeons (Chen 1965; Koek et al. 1984, 1986; Leander et al. 1986), exhibit antianxiety activity in some preclinical screens (cf. Sanger and Joly 1991; Wiley and Balster 1992), and have been reported to either partially or fully reproduce the discriminative stimulus effects of PCP-related drugs (cf. Balster and Willetts 1988; Willetts et al. 1990). Although the behavioral effects of competitive and noncompetitive ligands of the NMDA receptor can sometimes be distinguished (cf. Willetts et al. 1990), the considerable overlap in the behavioral pharmacology of these two classes of compounds has generally required labor- and time-intensive behavioral experiments to provide qualitative differentiation.

Since PCP and related compounds produce a complex spectrum of behaviors, drugs from a number of non-NMDA drug classes can also reproduce some of the behavioral effects of PCP-related compounds. Thus, certain serotonergic agonists can reproduce the head-weaving, ataxia, and locomotor effects of PCP (Martin et al. 1979; Lucki 1990). PCP and other uncompetitive NMDA antagonists also share the locomotor stimulatory and discriminative stimulus effects of classical psychomotor stimulants (Koek et al. 1989). Like CNS depressants, competitive and uncompetitive NMDA antagonists produce ataxia (cf. Tricklebank et al. 1989). Given the similarities in the pharmacological actions of strychnine-insensitive glycine receptor antagonists or partial agonists, polyamine antagonists, and σ ligands with the competitive and uncompetitive NMDA antagonists (e.g., anticonvulsant, neuroprotectant), overlap in behavioral effects has been anticipated.

Based on observations of the effects of PCP-related drugs on rotorod performance of rodents (Marwaha et al. 1981; Skolnick et al. 1989), Evoniuk et al. (1991) developed a behavioral test to distinguish the explosive behavior of PCP-treated animals from the ataxia and sedation also produced by other CNS depressants. In this test, mice were placed on a small circular platform with a raised edge (petri dish) that was suspended above the floor. A host of dissociative anesthetics produced a dose-dependent increase in the number of mice falling off the dish. Other CNS depressants, stimulants, or NMDA receptor ligands did not produce falling. Thus, this procedure provided a rapid behavioral differentiation of PCP-related drugs from other compounds. However, the Evoniuk procedure was less sensitive than other behavioral methods, with high doses of the dissociative anesthetics required to produce falling. The procedure also resulted in some false negatives with compounds displaying high affinity for the NMDA receptor ion channel ((+)-SKF 10,047 and the isomers of cyclazocine) being devoid of behavioral effects. In addition, some non-NMDA drugs tested positive (e.g., barbiturates). The present experiment was designed in an attempt to increase both the sensitivity and selectivity of the Evoniuk procedure without greatly altering the rapid nature of this drug screening device. A combination of objective behavioral tests that did not require animal or experimenter training was used to separately assess the ataxia and the locomotor activity inducing effects of these compounds. A comparable procedure was used to distinguish behavioral effects of PCP from those of the competitive NMDA antagonists, D(−)2-amino-5-phosphonovalerate (AP5) and DL-2-amino-7-phosphonoheptanoate (AP7) (Ornstein et al. 1987). In the present study, drugs from a variety of chemical and pharmacological classes were compared in order to evaluate the sensitivity and selectivity of these behavioral tests and to explore the relationship of these two behavioral components induced by dissociative anesthetics to those observed with other NMDA antagonists and non-related compounds.

Materials and methods

Behavioral procedure. Male Swiss Webster mice (Charles River) between 10 and 12 weeks old were housed five per cage in a temperature-controlled vivarium with a 12-h light/dark cycle. Behaviorally naive mice were studied and used only once, first in the inverted screen test (Coughenour et al. 1977) and then in a locomotor arena. After the appropriate pretreatment interval following drug injection, the mice were placed on a wire mesh screen measuring 14 × 14 cm (mesh width of 0.8 cm), 38 cm above the ground. The screen was slowly inverted and the mice were scored for their ability to climb back to the top of the screen, and thus be in an upright position. Failure to get all four paws onto the top of the screen within 2 min was scored as a screen failure. Immediately following their trial on the inverted screen they were placed in a 40 cm^3 Digiscan activity monitor equipped with photoelectric detectors placed 2.6 cm apart along the perimeter capable of sensing ambulatory activity at a height up to 20 mm above the floor (Omnitech Electronics, Columbia, Ohio). Activity levels were then recorded for 30 min.

Drugs. The following compounds were dissolved in distilled water: (±)-2-carboxypiperazine-4-yl-propyl-1-phosphonic acid [CPP, Research Biochemicals (RBI)], dextromethorphan HBr (RBI), dextrorphan D-tartrate (RBI), (±)-6-(1(2)H-tetrazol-5-yl)methyldecahydroisoquinoline-3-carboxylic acid (LY 233536, Lilly), (±)-(phosphonomethyl)-decahydroisoquinoline-3-carboxylic acid (LY 274614, Lilly), dizocilpine ((+)-MK-801, RBI), (−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10- imine hydrogen maleate ((−)-MK-801, RBI), NMDA (RBI), (±)-2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid (NPC 12626, Nova), pentylenetetrazol (PTZ, Sigma), (−)-quinpirole HCl (RBI), (−)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-*N*-m ethyl-5H-benzo[d]naptho-{2-1-b}azepine (SCH 39166, Schering Plough), 1-phenyl-2,3,4,5-tetrahydro-(1H)-3benzazapine-7,8-diol HCl (SKF 38393, RBI), (±)-8-hydroxydipropylaminotetralin HBr (8-OH-DPAT, RBI) and NBQX (Novo Nordisk). 7-Chlorokynurenic acid (RBI) was dissolved in distilled water with the minimal NaOH and ifenprodil (Synthelabo) and (±)-α −4-chlorophenyl(4-fluorophenylmethyl)-4 piperidine-1-ethanol HCL (SL 82.0715–10, Syn-

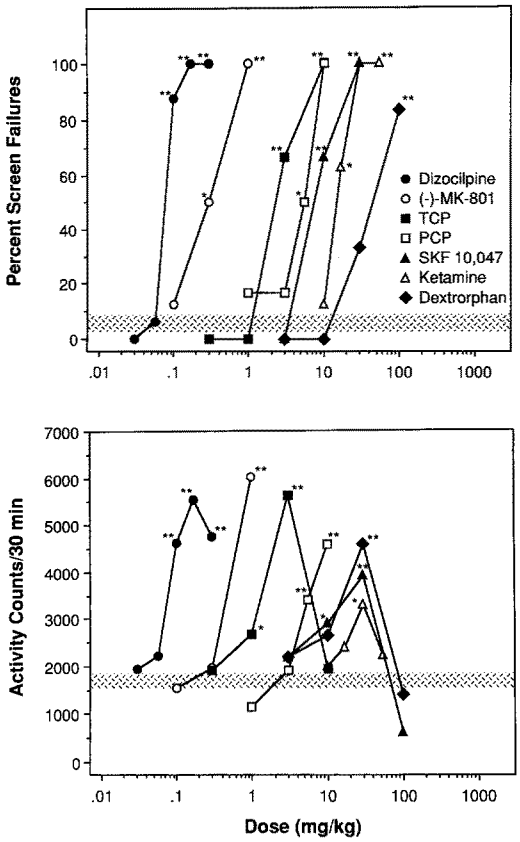

Fig. 1. Effects of uncompetitive NMDA antagonists on the inverted screen test (*top panel*) and on locomotor activity (*bottom panel*). Each point represents effects in at least six mice. Shaded regions represent ± SEM of control values. Statistical comparisons with control values were determined using two-tailed Dunnett's test (locomotor activity) or Fisher's Exact Probability test (inverted screen). $*P < 0.05$; $**P < 0.01$

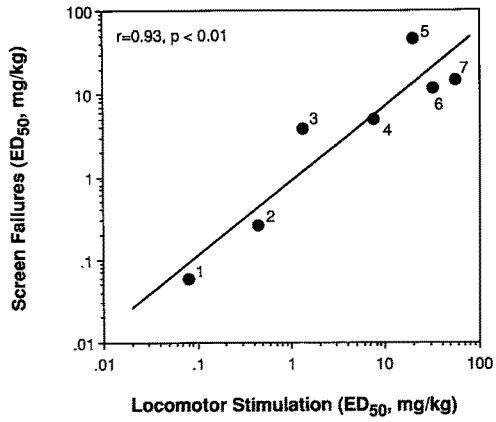

Fig. 2. Relationship between ED_{50} values for producing failures in the inverted screen test and producing increases in locomotor activity. *Numbers* refer to compounds in Table 1

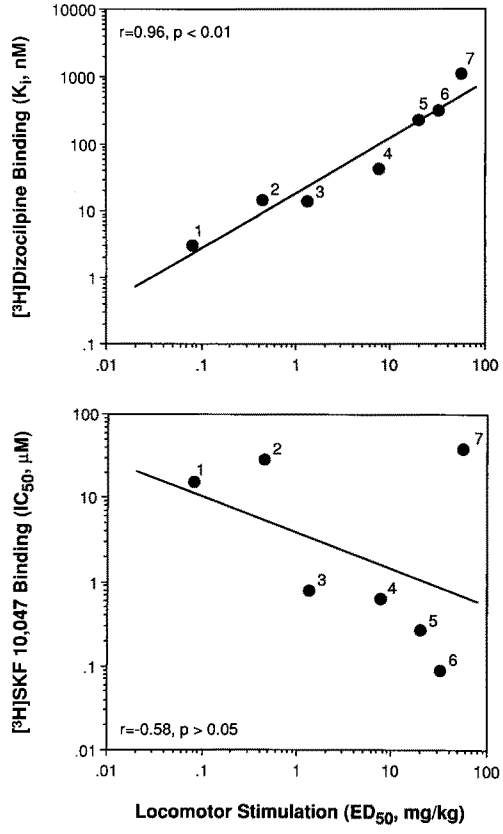

Fig. 3. Relationship between the ED_{50} values for producing failures in the inverted screen test and affinities for PCP receptors ([^{3}H]dizocilpine) or σ receptors ([^{3}H]SKF 10,1047). *Numbers* refer to compounds in Table 1

thelabo), 1,3-di(2-tolyl)guanidine (DTG, RBI), and haloperidol (McNeil), with the minimal HCl required for solution with mild heat and sonification. Ketamine HCl (Sigma), PCP HCl (National Institute on Drug Abuse), 1-[1-(2-thienyl)-cyclohexyl]piperidine HCl (TCP, National Institute on Drug Abuse), pentobarbital Na (Abbott), phenobarbital Na (Ruger), (−)-cocaine HCl (Mallinckrodt/Nuclear), and (+)-methamphetamine HCl (S) were dissolved in 0.9% NaCl. Diazepam (Hoffmann La-Roche) was suspended in 20% propylene glycol. Drug doses are expressed as the drug forms noted above. All drugs were given by SC injection except 7-chlorokynurenic acid, NBQX, quinpirole, and SKF 38393 which were given IP. Routes of administration and pretreatment times were chosen on the basis of demonstrated anticonvulsant efficacy (cf. Tortella et al. 1992) and pilot experiments. Drugs were given 30 min prior to testing except ketamine (10 min), 8-OH-DPAT (10 min), SKF 38393 (15 min), quinpirole (15 min), and NBQX (15 min). Injection volumes were generally 0.01 ml/g body weight although higher doses of some of the drugs were given in twice the volume.

Data analysis. Dose-effect functions for locomotor activity were analyzed using data from the linear portion of the curves using standard bioassay analysis of variance (ANOVA) techniques (Finney 1964; Snedecor and Cochran 1967). Individual contrasts were evaluated with two-tailed Dunnett's test. ED_{50} values for locomotor activity were determined by assuming maximal stimulation to occur at the peak of the dose-effect curve for the most efficacious compound. The 50% level of stimulation was then fixed as midway between control values (vehicle) and this peak effect. Quantal data on screen failures were evaluated according to the methods described by Litchfield and Wilcoxon (1949) with specific comparisons between treatments with Fisher's Exact Probability test. Statistical probabilities of greater than 0.05 were considered to be non-significant.

Results

Compounds which bind noncompetitively and with high affinity to the NMDA receptor-associated ion channel

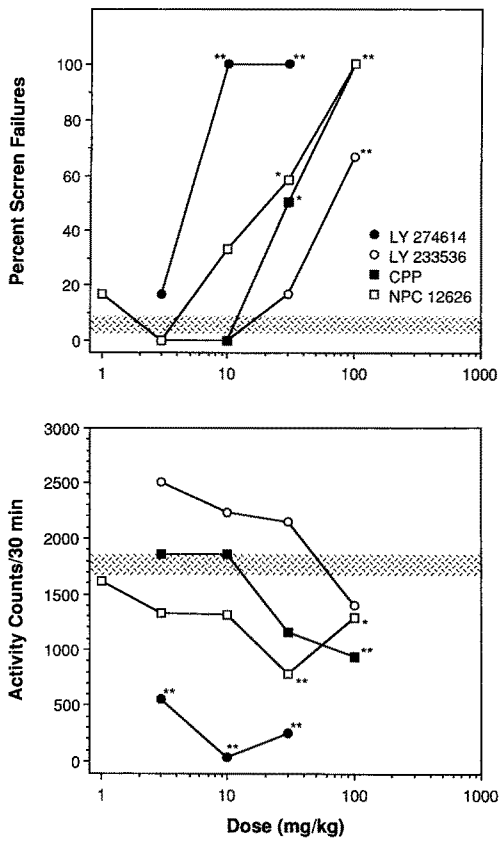

Fig. 4. Effects of competitive NMDA antagonists on the inverted screen test (*top panel*) and on locomotor activity (*bottom panel*). Other details as in Fig. 1

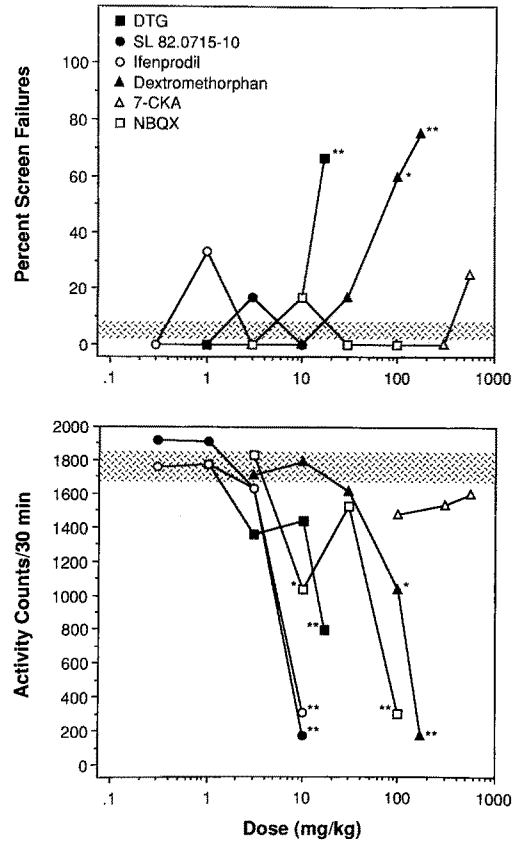

Fig. 5. Effects of σ ligands (*DTG*, dextromethorphan), polyamine ligands (ifenprodil, SL 82.0715–10), a strychnine-insensitive glycine antagonist (7-chlorokynorenic acid), and an AMPA antagonist (*NBQX*) on the inverted screen test (*top panel*) and on locomotor activity (*bottom panel*). Other details as in Fig. 1

Table 1. Comparison of ED_{50} Values (95% confidence limits) for Locomotor Stimulation and Failure on the Inverted Screen Test with affinities for PCP ([^{3}H]Dizocilpine) or Sigma Receptors ([^{3}H]SKF 10,1047)[1].

Compound	Locomotor activity	Screen test	[^{3}H]Dizocilpine (K_i, nM)	[^{3}H]SKF 10,047 (IC_{50}, uM)
1. Dizocilpine	0.08 (0.07–0.1)	0.06 (0.06–0.09)	3	14.9
2. (−)-MK-801	0.45 (0.37–0.54)	0.26 (0.12–0.55)	15	28.2
3. TCP	1.32 (1.05–1.66)	3.82 (1.99–7.34)	14	0.79
4. PCP	7.70 (5.05–11.7)	5.00 (2.94–8.49)	42	0.63
5. Dextrorphan	20.1 (9.95–40.5)	46.7 (20.7–105)	222	0.27
6. (+)-SKF 10,047	31.95 (5.64–48.8)	11.8 (6.22–22.4)	317	0.09
7. Ketamine	55.2 (10.9–278)	14.7 (10.8–20.0)	1090	38.4

Binding data are from Wong et al. (1988)

(see Table 1) all produced dose-related increases in screen failures and locomotor activity at comparable doses (Fig. 1). Some of these drugs displayed inverted U-shaped dose-effect curves for locomotor activity with higher doses producing less of an increase than moderate doses. Peak increases in locomotor activity occurred at doses that significantly increased failures on the screen test and were of the order of 2.5–3.5 times control levels of activity. Ketamine produced the smallest degree of locomotor stimulation when given 10 min prior to testing as shown in Fig. 1, but did not significantly stimulate locomotion when given 30 min prior to testing (not shown). Potencies of these compounds for producing screen failures correlated highly with locomotor stimulatory effects; the correlation coefficient for this relationship was highly significant ($r = 0.93$, $P < 0.01$) (Fig. 2). The regression equation, $y = 0.9x - 0.05$, indicates further that both locomotor stimulation and screen failures occurred at approximately the same point on the dose-effect function.

A significant positive correlation between the ED_{50}

[1] The facilities in which the animals were maintained are fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and the studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the NIH and adopted by NIDA.

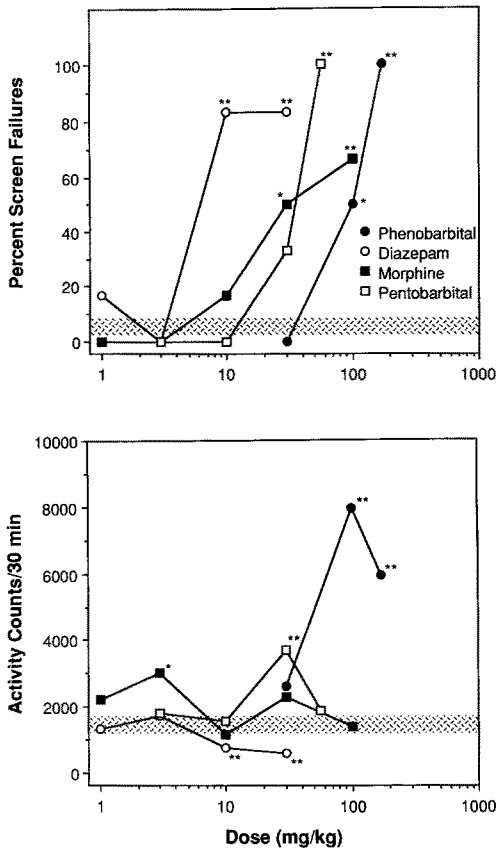

Fig. 6. Effects of some CNS depressants on the inverted screen test (*top panel*) and on locomotor activity (*bottom panel*). Other details as in Fig. 1

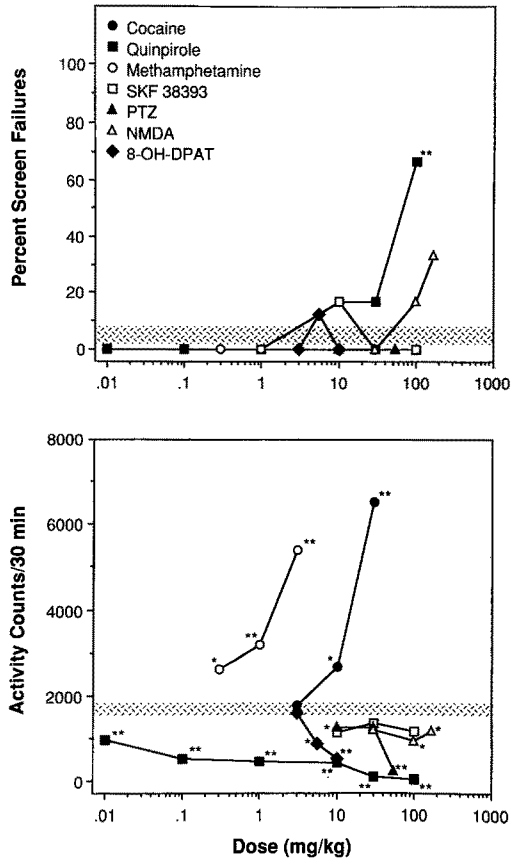

Fig. 7. Effects of some dopamine and serotonin agonists and convulsants on the inverted screen test (*top panel*) and on locomotor activity (*bottom panel*). Other details as in Fig. 1

values for these behavioral effects and affinities of the compounds for [^{3}H]dizocilpine binding sites was observed ($r = 0.85$, $P < 0.05$ for screen failures versus [^{3}H]dizocilpine binding affinities; $r = 0.96$, $P < 0.01$ for locomotor activity versus [^{3}H]dizocilpine binding affinities). Since locomotor activity and screen failures were positively related (Fig. 2) the relationship for locomotor activity only is shown for illustrative purposes (Fig. 3). No correlation was observed between either of these behavioral effects of uncompetitive NMDA antagonists and affinities for σ receptors as measured with [^{3}H]SKF 10,047 binding (Fig. 3). The correlation coefficient for the relationship between potencies in producing screen failures and [^{3}H]SKF 10,047 binding affinities was $r = -0.43$, $P > 0.05$; the correlation coefficient for locomotor activation versus [^{3}H]SKF 10,047 binding was $r = -0.58$, $P > 0.05$.

The competitive NMDA antagonists LY 274614, LY 233536, CPP, and NPC 12626 all produced dose-dependent increases in screen failures with a rank order potency of LY 274614 > NPC 12626 = CPP > LY 23356 (Fig. 4). Whereas LY 23356 did not produce full effect on the screen test up to a dose of 100 mg/kg or produce any effects on locomotor activity, the other compounds were fully efficacious on the screen test and produced decreases in locomotor activity. Whereas NPC 12626 and CPP produced significant effects on locomotor activity and on the screen test at about the same doses, LY 274614 produced a profound decrease in locomotor activity at 3 mg/kg, a dose which did not significantly increase screen failures.

The polyamine ligands ifenprodil and SL 82.0715–10 did not significantly alter the behavior of mice on the screen test but both compounds decreased locomotor activity at 10 mg/kg (Fig. 5, circles). The σ ligands DTG and dextromethorphan both increased screen failures and decreased locomotor activity at about the same doses (Fig. 5). 7-Chlorokynurenic acid, an antagonist of the strychnine-insensitive glycine receptor, did not significantly affect performance on either the screen test or in the locomotor activity arena (Fig. 5). Although the AMPA receptor antagonist NBQX did not alter behavior on the screen test, decreases in locomotor activity were observed at 10 and 100 mg/kg (Fig. 5).

All of the general CNS depressants tested increased the percentage of screen failures (Fig. 6). Diazepam also decreased locomotor activity at doses that significantly affected performance on the screen test whereas morphine produced small increases in locomotor activity at doses that did not alter behavior on the screen test; even high doses of morphine did not decrease locomotion. Both barbiturates increased locomotor activity, with phenobarbital producing exceptional stimulation (Fig. 6).

Of the dopamine agonists tested, only the D_2 receptor agonist quinpirole increased screen failures. Nonetheless,

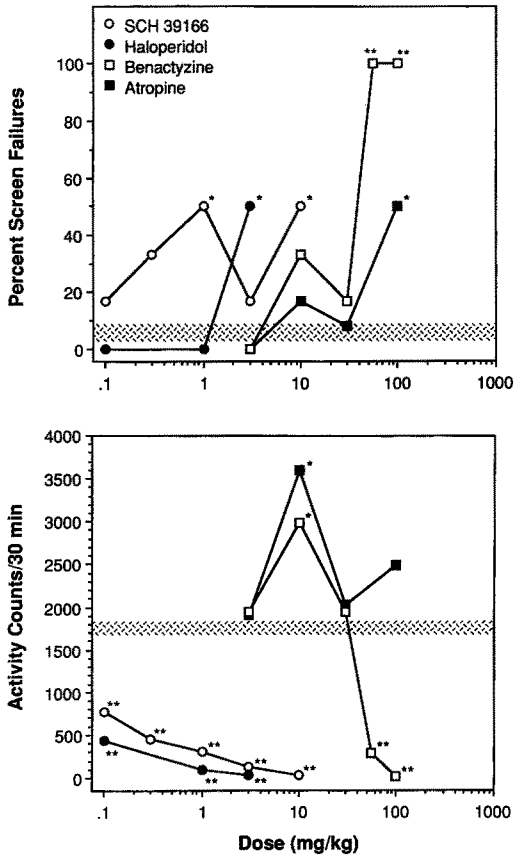

Fig. 8. Effects of dopamine antagonists and muscarinic antagonists on the inverted screen test (*top panel*) and on locomotor activity (*bottom panel*). Other details as in Fig. 1

both quinpirole and the D_1 receptor partial agonist SKF 38393 also decreased locomotor activity; quinpirole produced large decreases in activity at doses 4 orders of magnitude lower than those affecting screen test performance (Fig. 7). Both indirect-acting dopamine agonists, cocaine and methamphetamine, produced dose-related stimulation of locomotion (Fig. 7). Whereas the convulsants PTZ and NMDA failed to affect performance on the screen test, both compounds produced decreased levels of locomotor activity (Fig. 7). The serotonin ($5HT_{1A}$) agonist 8-OH-DPAT also produced dose-related decreases in locomotor activity.

Antagonists of both dopamine D_1 (SCH 39166) and D_2 (haloperidol) receptors increased screen failures to a maximum of only 50% while profoundly suppressing locomotor activity even at doses without activity in the screen test (Fig. 8). The muscarinic cholinergic antagonists increased screen failures, with benactyzine being fully efficacious. Both atropine and benactyzine also stimulated locomotor activity but increases were only 2-fold or less and occurred at doses 1 order of magnitude lower than those affecting screen performance (Fig. 8).

Discussion

The present study demonstrated that the dose-dependent increases in locomotor activity and failures on an inverted screen test could be used to provide a rapid and sensitive method for distinguishing PCP-type noncompetitive NMDA antagonists from other classes of centrally acting drugs. For this class of compounds, potencies in one behavioral assay were predictive of potencies in the other (Fig. 2). The procedure is rapid and requires no training of either the experimental subjects or the experimenters as is typically required in other behavioral assessments. Moreover, in contrast to some observational methods, the present procedure provides objective measures of behavior that are unambiguously observed and recorded and not subject to specific rating scales. The present procedure was also very sensitive. ED_{50} values for effects for locomotor stimulation and screen failures were 1.3–12.5 times lower than the minimal effective doses reported in the elevated platform test by Evoniuk et al. (1991). Further, the doses effective in producing behavioral effects in the present study were within the range of doses active *in vivo* against NMDA-induced lethality or behavioral effects (Leander et al. 1988; Koek et al. 1990), in stimulating schedule-controlled responding (Sanger and Jackson 1989; Genovese and Lu 1991) as anticonvulsants (Ferkany et al. 1989; Koek et al. 1990; Witkin and Tortella 1991), or in producing PCP- or dizocilpine-like discriminative stimulus effects (cf. Holtzman 1980; Balster et al. 1988; Iversen et al. 1988; Koek et al. 1990; Witkin and Steele 1992).

The behavioral effects of the uncompetitive NMDA antagonists were stereoselective with dizocilpine 4.3–5.6 times more potent that its enantiomer (−)-MK-801. These potency differences correspond to the 5-fold difference in affinities for [^{3}H]dizocilpine binding sites (Table 1). Behavioral effects of the uncompetitive NMDA antagonists were directly related to their affinities for PCP receptors (Zukin and Zukin 1979; Wong et al. 1988) as shown in Fig. 3. Other behavioral effects of PCP and related compounds have also been observed at doses consistent with their affinities for the PCP receptor (cf. Balster and Willetts 1988; Koek and Woods 1988; Willetts and Balster 1988).

Although a number of the uncompetitive NMDA antagonists bind to σ receptors, σ receptor binding was not associated with the behavioral effects observed here (Fig. 3). Further, the σ ligands haloperidol, DTG, ifenprodil, and dextromethorphan did not produce behavioral effects like the uncompetitive NMDA antagonists (Figs. 5, 8). The NMDA antagonist actions of these compounds predominated even when affinities for σ receptors were greater than [(+)-SKF 10,047] or equal to (dextrorphan) affinities at PCP receptors. These data correspond to observations that both (+)-SKF 10,047 and dextrorphan substitute for the discriminative stimulus effect of PCP (Holtzman 1980; Brady et al. 1982; Shannon 1982; Jackson and Sanger 1988). That (+)-SKF 10,047 did not produce PCP-like effects in the elevated platform test (Evoniuk et al. 1991) suggests that the present behavioral methods may be more efficient in detecting compounds with PCP-like behavioral effects.

Behavioral effects of other classes of ligands which interact with the NMDA receptor-ionophore complex did not reproduce the spectrum of behavioral effects ob-

served with the uncompetitive antagonists. This was particularly striking with the competitive NMDA antagonists whose behavioral effects often overlap with those of the uncompetitive ligands (see Introduction). The present data (see also Ornstein et al. 1987; Evoniuk et al. 1991) emphasize differences in the behavioral effects of competitive and uncompetitive antagonists of the NMDA receptor that have increasingly been recognized (cf. Willetts et al. 1990). However, it is possible that some of these apparent differences may be related to the generally poorer CNS penetrability of some of the competitive NMDA antagonists. Differences in the behavioral activities among competitive antagonists could also be discerned from this simple behavioral method (Fig. 4). For example, LY 274614 profoundly suppressed locomotion at doses inactive in the screen test whereas the structurally related compound LY 233536 did not significantly alter locomotor activity at doses up to 100 mg/kg. That the AMPA antagonist NBQX was also not PCP-like in these tests suggests that blockade of excitatory amino acid neurotransmission per se is not sufficient for the induction of PCP-like behavioral effects. Results from clinical investigations with AMPA antagonists and competitive NMDA antagonists will be required to establish the preclinical information sufficient for predicting the side-effect profile of these compounds.

The polyamine ligands ifenprodil and SL 82.0715–10 and an antagonist of the strychnine-insensitive glycine receptor, 7-chlorokynurenic acid, also did not exhibit PCP-like behavioral effects in this experiment (Fig. 5). Evoniuk et al. (1991) noted a similar absence of effects for the strychnine-insensitive glycine ligands 1-aminocylopropane-carboxylic acid (ACPC) and 7-chlorokynurenic acid in the elevated platform test. Further evaluation of a series of glycine antagonists will be necessary to confirm the pharmacological generality of these findings, especially in light of apparent differences in behavioral effects of these compounds (cf. Bourson and Tricklebank 1991; Koek and Colpaert 1992). Experiments using observational methods with 7-chlorokynurenic acid and ifenprodil have also not detected falling and locomotion (Koek and Colpaert 1990). Despite generally comparable pharmacological profiles (e.g., anticonvulsant, neuroprotectant), differences in the behavioral effects of these drugs and PCP-like compounds have also been revealed in more complex behavioral tests. Thus 7-chlorokynurenic acid, ACPC and ifenprodil do not substitute for the discriminative stimulus effects of PCP or dizocilpine (Jackson and Sanger 1988; Witkin and Steele 1992). This information, combined with the absence of notable overt behavioral effects with 7-chlorokynurinc acid or ACPC (Fig. 5; Skolnick et al. 1989; Evoniuk et al. 1991; Witkin and Tortella 1991), suggests a large therapeutic window for the clinical application (cf. Carter 1992) of this drug class.

The behavioral effects of phencyclidine and other uncompetitive NMDA antagonists, like dizocilpine, also overlap substantially with classical psychomotor stimulant compounds (see Introduction). Although cocaine and methamphetamine reproduced the locomotor stimulant effects of the uncompetitive NMDA antagonists, these compounds had no effect in the inverted screen test, demonstrating that behavioral stimulant effects alone are insufficient to engender the PCP-behavioral syndrome (Fig. 7). Direct acting dopamine stimulants, acting either at D_1 or D_2 receptors, also failed to produce PCP-like behavioral effects. Thus, although catecholamines have been implicated in the locomotor stimulant effects of PCP and related compounds (Chen et al. 1959; Clineschmidt et al. 1982; Irifune et al. 1991; Löscher and Hönack 1992), these effects do not appear sufficient to account for this behavioral effect of uncompetitive NMDA antagonists (see also Carlsson and Carlsson 1989). The differences in the behavioral effects of direct and indirect acting dopamine agonists observed here (Fig. 7) further suggest the utility of this method for differentiating behavioral effects of other classes of psychoactive drugs. Some of the behavioral effects of PCP and related drugs can also be mimicked by certain serotonergic agonists and blocked by antagonists (Martin et al. 1979; Löscher and Hönack 1992). Although the $5HT_{1A}$ agonist 8-OH-DPAT can induce, under some conditions, the head-weaving, locomotor stimulation, and ataxia also seen with PCP (cf. Lucki 1990), 8-OH-DPAT was not PCP-like in its behavioral effects in the present study, providing further evidence for the sensitivity of the current behavioral method.

Antimuscarinic antagonists have been known to produce psychotomimetic effects in humans that resemble to some extent the symptoms of PCP intoxication (cf. Abood and Biel 1962; Ketchum et al. 1973). Like PCP, benactyzine and atropine produced increases in both locomotor activity and screen failures (Fig. 8). Nonetheless, the behavioral effects were distinguished from those of the uncompetitive NMDA antagonists in the small magnitude of locomotor stimulation produced by these antimuscarinics as previously reported (Witkin et al. 1992) and the 10-fold separation in their locomotor stimulant versus screen performance impairing effects.

The barbiturates, pentobarbital and phenobarbital, produced effects that were comparable with those of the uncompetitive NMDA antagonists (Fig. 6) and thus constitute the only false positives with this behavioral test. Large dose-dependent increases in locomotor activity have been observed previously with some barbiturates (e.g., Waters and Walczak 1980). Thus, the pigeon catelepsy test and drug discrimination methods appear to more effectively differentiate behavioral effects of barbiturates from PCP-type noncompetitive NMDA antagonists. CNS depressant effects per se of these compounds were not sufficient to induce PCP-like behavioral effects as other CNS depressants including morphine, diazepam, haloperidol, and SCH 39166 were not PCP-like (Figs. 6, 8). Under other conditions, behavioral effects of barbiturates show a more remarkable overlap with the behavioral effects of competitive NMDA antagonists. Thus, at least partial substitution between the discriminative stimulus effects of these classes of compounds has been observed (Willetts and Balster 1989; Willetts et al. 1989, 1991). Although Evoniuk et al. (1991) also reported barbiturates as false positives in the elevated platform test, the nature of the common behavioral effects of these bar-

biturates and uncompetitive NMDA antagonists is not clear. Barbiturates and PCP share a number of CNS depressant actions (Balster and Wessinger 1983). However, evidence suggests that barbiturates typically show greater efficacy against quisqualate- and kainate-induced neuronal excitation than observed against NMDA (Teichberg et al. 1984; Collins and Anson 1987; Addae and Stone 1988; Lodge and Johnson 1990). Nonetheless, anticonvulsant actions of dizocilpine can be potentiated by both pentobarbital and phenobarbital (Kulkarni and Ticku 1989) that may be related to the reported inhibitory effects of barbiturates on glutamatergic transmission (Macdonald and Barker 1979). Thus, whether the common pharmacological effects of uncompetitive NMDA antagonists and barbiturates represent independent or interacting neuronal processes is not presently known.

In summary, the present behavioral method constitutes a sensitive and rapid method for differentiating the behavioral effects of PCP-type noncompetitive NMDA antagonists which correlates highly with binding affinities for the NMDA receptor ion channel. The results demonstrate that the objective measurement of two of the important behavioral components of action of PCP-like drugs (locomotor stimulation and ataxia/sedation) may be useful for detecting PCP-like behavioral effects in newly developed compounds. For example, ADCI, a dizocilpine analog with low affinity for NMDA receptors, retains anticonvulsant efficacy without producing the PCP-like side effects described here (Seidleck et al. 1994). These methods should also be well suited for investigation of the influence of ligands acting outside the NMDA cation channel on the behavioral effects of PCP-like drugs (cf. Downs et al. 1988).

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