ELSEVIER

European Journal of Pharmacology 367 (1999) 1–5

ejp

Short communication

Increased discriminative stimulus potency of phencyclidine in C57Bl/6 mice infected with the LP-BM5 retrovirus

Justin A. English, Karen H. Bruce, Ian A. Paul *

Laboratory of Neurobehavioral Pharmacology and Immunology, Division of Neurobiology and Behavior Research, Department of Psychiatry—Box 127, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216-4505, USA

Received 16 December 1998; accepted 22 December 1998

Abstract

Drug discrimination procedures in mice are used to study the neuropharmacology of a wide variety of drugs. In C57 Bl/6 mice, infection with the LP-BM5 murine leukemia virus leads to a syndrome (murine acquired immunodeficiency syndrome-MAIDS) characterized by immunocompromise, neurochemical alterations, and learning and memory deficits. Because the neurochemical and behavioral changes suggest that altered glutamatergic neurotransmission follows LP-BM5 infection, we studied the effects of infection on discriminative stimulus properties of phencyclidine (PCP), a Ca^{2+} channel blocker at NMDA receptors. We also tested D-amphetamine and dizocilpine to assess the specificity of the discrimination. As expected, dizocilpine produced PCP-like responding. After animals were trained to discriminate PCP from saline, they were inoculated with LP-BM5 and the PCP dose-response functions repeatedly determined. The potency of PCP in this procedure was unchanged 3 weeks after infection, but was increased approximately fivefold 6 and 9 weeks after infection. Amphetamine 9 weeks after inoculation did not produce PCP-like responding, showing that the results were not caused by a loss of specificity of the discrimination. The time course for changes in PCP potency is similar to those of other behavioral and neurochemical changes reported after LP-BM5 infection. The results are consistent with an action of LP-BM5 infection at glutamatergic synapses.

Keywords: LP-BM5; Retrovirus; Drug discrimination; Phencyclidine; NMDA; MAIDS

1. Introduction

Drug discrimination procedures in animals are used to predict subjective effects of psychoactive drugs, and to investigate neuropharmacology of those effects. Mice reliably discriminate several classes of abused drugs, including stimulants (Snoddy and Tessel, 1983), barbiturates (Balster and Moser, 1987), organic solvents (Rees et al., 1985), opioids (Borlongan and Watanabe, 1995), and inhalant anesthetics (Rees et al., 1987). Discriminative stimulus effects of phencyclidine (PCP) have been studied extensively (Balster, 1991), and are linked to blockade of Ca^{2+} entry at NMDA receptors (Martin and Lodge, 1988; Lodge and Johnson, 1990; Willets et al., 1990). NMDA receptors are particularly concentrated in rodent cortex and hippocampus (Cotman et al., 1987), and are important in spatial memory and long-term potentiation (Butcher et al., 1990; Butcher et al., 1991; Davis et al., 1992).

In C57Bl/6 mice, inoculation with the LP-BM5 murine leukemia viruses causes immunologic abnormalities including lymphadenopathy, splenomegaly, disrupted T- and B-cell response to mitogenic and antigenic stimuli (Mosier et al., 1989), polyclonal B-cell activation (Klinman and Morse, 1989), decreased resistance to infection (Buller et al., 1987), and B-cell lymphomas (Klinken et al., 1988). This syndrome is referred to as murine acquired immune deficiency syndrome or MAIDS (Hartley et al., 1989; Jolicoeur, 1991). In later stages, MAIDS leads to neurological symptoms such as hindlimb paralysis, ataxia and generalized tremor (Buller et al., 1987), paralleling neurological symptoms associated with late-stage AIDS (Navia et al., 1986). MAIDS also impairs learning in the Morris water maze, a measure of spatial learning (Sei et al., 1992a), and causes a deficit in acquisition, but not performance, of avoidance responding in a shuttle shock-avoidance procedure (English et al., 1998).

LP-BM5 infects the central nervous system (Sei et al., 1992b), and causes a 10-fold increase in CNS concentrations of quinolinic acid (Sei et al., 1996), an excitotoxic

* Corresponding author. Tel.: +1-601-984-5998; Fax: +1-601-984-5884; E-mail: ipaul@psychiatry.umsmed.edu

0014-2999/99/$ - see front matter
PII: S0014-2999(98)00961-3

tryptophan metabolite. Because behavioral and neurochemical changes after LP-BM5 infection suggest alterations in glutamatergic neurotransmission, we hypothesized that LP-BM5 infection would alter the effects of PCP in a drug discrimination procedure. As a positive control, we tested dizocilpine, a compound that produces PCP-like responding in discrimination procedures (Willetts and Balster, 1988; Geter-Douglass and Witkin, 1997). We tested D-amphetamine as a negative control 9 weeks after inoculation to assess whether the effects of PCP would generalize to another compound with locomotor stimulant activity, but a different pharmacological mechanism. LP-BM5 infection time-dependently increased the potency of PCP as a discriminative stimulus, without affecting the specificity of the discrimination or other nonspecific effects as indicated by rate of responding.

2. Materials and methods

2.1. Animals

Male C57Bl/6 mice (Jackson Labs) were group housed (5 animals per group at the start of the experiment) with a 12:12 h light–dark cycle (lights on at 07:00) and ad libitum food access. They were water-restricted approximately 23 h/day.

2.2. Training procedure

Training took place in operant chambers equipped with three nose poke units on one wall, a wire grid floor, and a plexiglas door. A dipper delivering water could be accessed in the center unit (Med. Associates, St. Albans, VT). Chambers were located in sound and light-attenuated enclosures (Coleman Industries, Golden, CO).

Mice were trained five days per week in sessions that lasted 30 min or until 1000 responses or 100 dipper operations occurred. Mice were first trained in the nose-poke response, then trained to poke either the right or left unit when given drug and the alternative when given vehicle. Correct responses raised the dipper for 5 s. Incorrect responses turned off the house light and began a 2 s timeout. Responses during the timeout reset the timer to 2 s. Mice were run with a fixed ratio-1 (FR1) schedule. Chambers were swabbed with 10% ethanol solution between subjects to remove olfactory cues. After each session, animals had free access to water for 1 h.

Drug administration was counterbalanced in a two-week cycle with the sequence SDDSS and DSSDD, where D is drug and S saline. When responding was at criterion (80% or more condition-appropriate responding), test sessions were introduced using the sequences SDTST and DSTDT, where T is a test session. Test sessions were like training sessions, except that both side units operated the dipper and no timeout was associated with either side. In order to achieve, test and retest criterion, mice were exposed to PCP at varying doses 3–5 times per week over six months.

In this preliminary study, we were most concerned that the response to PCP was due to activity at the NMDA receptor coupled ionophore and was not an artifact of psychomotor stimulation. Thus, we compared the response to PCP to the response to another ionophore antagonist (dizocilpine) and a pharmacologically distinct motor stimulant (D-amphetamine) with little or no direct activity at the NMDA receptor.

When animals made more than 80% condition-appropriate responses under test conditions with the training dose, the dose–response curve for PCP and dizocilpine was determined. Animals were then inoculated and PCP dose-response was redetermined approximately 3, 6, and 9 weeks post-inoculation. The effect of D-amphetamine was determined approximately 9 weeks post-inoculation.

2.3. Drugs

All drugs were given i.p. in saline in an injection volume of 0.2 ml. Doses of phencyclidine hydrochloride (RBI, Natick, MA) ranged from 0.1 to 6.0 mg/kg. Dizocilpine hydrogen maleate (RBI, Natick, MA) doses were 0.01 to 0.1 mg/kg. *S*(+)-amphetamine sulfate (RBI, Natick, MA) was tested at 6.0 mg/kg. Drugs were administered 5 min before the session.

2.4. LP-BM5 inoculation

When animals made more than 80% condition-appropriate responses under test conditions with the training dose, over a six week period, they were inoculated (i.p.) with 0.1 ml of LP-BM5 MuLVs containing $10^{4.5-5.6}$ XC plaque forming units (PFUs) per ml of ecotropic and $10^{2.5-3.2}$ mink cell focus (MCF) forming units per ml of MCF. At the conclusion of study, all animals were killed by cervical dislocation and decapitation. The brains and spleens were dissected. The spleens were weighed to assess splenomegaly (spleen weight > 100 mg) and the brains processed according to the methods described by Hartley et al. to assess viral titer (Hartley et al., 1989).

2.5. Data analysis and statistics

We calculated individual ED_{50}s for PCP to produce 80% drug-appropriate responding at 0, 3, 6, and 9 weeks post-inoculation. Data were analyzed with repeated-measures analysis of variance (ANOVA), followed by paired-observations *t*-tests to assess differences in potency at specific time points. Thus, each animal's baseline (uninoculated) performance served as the control for the effects of LP-BM5 inoculation on performance. An alpha level of 0.05 was used for statistical tests. We also calculated response rate in the first 30 s, maximum percent PCP-appropriate responding for each drug, and ED_{50}s at 0, 3, 6,

and 9 weeks post-inoculation. Response rates were analyzed with the Friedman test.

The number of possible testing days over any two-week interval post-inoculation was limited to four and drug sensitivity increased with time after infection. Thus, it was necessary to reduce the dose range of PCP tested over the course of the experiment. Since these constraints resulted in an incomplete blocks design, data were analyzed in two difference ways in order to assess effects of PCP dose and weeks post-inoculation. In the first analysis, a limited set of drug treatments (saline and 1 mg/kg PCP) were analyzed over all nine weeks post-inoculation. In the second analysis, a broader range of drug treatments (0, 1, 3 and 6 mg/kg PCP) were analyzed over a limited time post-inoculation (0, 3 and 6 weeks).

3. Results

LP-BM5 infection time-dependently increased the potency of PCP as a discriminative stimulus. Repeated-measures ANOVA showed a significant changes in the potency of PCP over time ($F(3,15) = 3.44,\ P < 0.05$). Potency was unchanged 3 weeks after inoculation, but was increased approximately 5-fold at 6 and 9 weeks after inoculation (Fig. 1). Mean ED_{50} before inoculation was 4.5 ± 1.0 mg/kg. At three weeks post-inoculation it was 4.5 ± 1.7 mg/kg. ED_{50} decreased to 0.5 ± 0.2 mg/kg and 1.3 ± 1.1 mg/kg six and nine weeks post-inoculation. ED_{50}s did not differ significantly between baseline and three weeks ($t_5 = 0.008,\ P = 0.99$), but were significantly different between baseline and six ($t_5 = 4.79,\ P = 0.005$), and baseline and nine weeks ($t_5 = 4.35,\ P = 0.001$). PCP did not reduce rate significantly compared to saline rates during any of the dose–response determinations.

As expected, dizocilpine produced substantial PCP-appropriate responding, and was about 7 times as potent as PCP, with an ED_{50} of 0.3 mg/kg indicating that the discriminative cue for PCP was based on activity at the NMDA receptor. Amphetamine (6 mg/kg) did not produce substantial PCP-appropriate responding, (maximum PCP responding $= 5.0 \pm 1.4\%$), but decreased the rate of responding ($X^2 = 6.34,\ P < 0.03$) indicating that the increased sensitivity to PCP was not a function of a generalized psychomotor activation. Neither PCP nor dizocilpine affected rate.

Saline response rates decreased after animals were inoculated ($X^2 = 11.00,\ P < 0.0005$), but rates for the 1.0, 3.0, and 6.0 PCP doses did not change after inoculation. Analy-

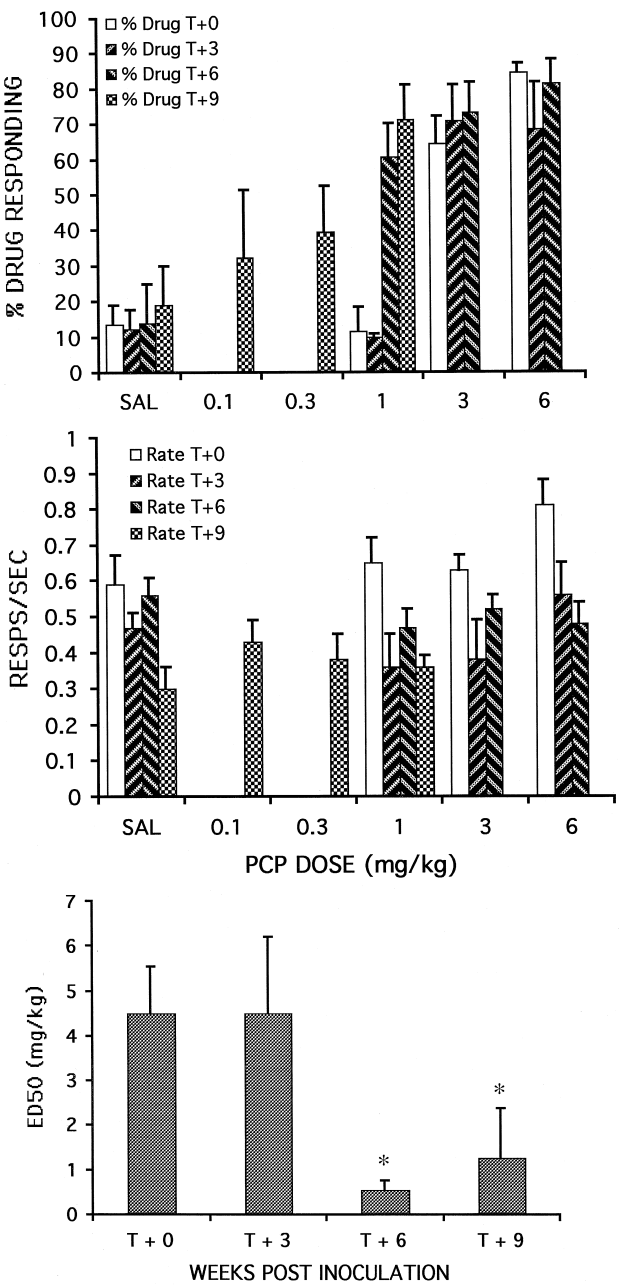

Fig. 1. Effects of LP-BM5 infection on percent drug appropriate responding when dose–response functions for PCP were determined immediately before inoculation with LP-BM5, and three, six, and nine weeks after inoculation. Doses were 1.0, 3.0, and 6.0 mg/kg before infection and 3 and 6 weeks after infection; at 9 weeks doses were 0.1, 0.3, and 1.0 mg/kg. Data represent the mean ± S.E.M. of 6 mice. Upper panel: Percent drug appropriate responding. Single factor (saline vs. 1 mg/kg PCP) repeated measures (0, 3, 6 ans 9 weeks post-inoculation) ANOVA revealed significant main effects of dose [$F_{1,5} = 11.642,\ p = 0.019$], weeks post-inoculation [$F_{3,15} = 9.537,\ p = 0.001$] and an interaction between dose and weeks post-inoculation [$F_{3,15} = 6.511,\ p = 0.005$]. Likewise, single factor (saline vs. 1, 3 and 6 mg/kg PCP) repeated measures (0, 3, and 6 weeks post-inoculation) ANOVA revealed significant main effects of dose [$F_{3,15} = 64.113,\ p < 0.0001$], weeks post-inoculation [$F_{2,10} = 9.968,\ p = 0.004$] and an interaction between dose and weeks post-inoculation [$F_{6,30} = 3.124,\ p = 0.017$]. Middle panel: Response rate during testing. No significant effects were observed across PCP dose or weeks post-inoculation. Lower panel: Summary of calculated ED_{50} values at baseline (T + 0) and 3, 6 and 9 weeks post-inoculation (T + 3, T + 6, and T + 9, respectively). Single factor (weeks post-inoculation) ANOVA revealed a significant overall effect [$F_{3,15} = 3.435,\ p = 0.044$]. $*\ p < 0.005$ in paired uncorrected Student's t-test.

sis of the saline data with Dunn's multiple comparison test showed that saline response rates were lower at 9 weeks than they were pre-inoculation or at 3 weeks post-inoculation.

4. Discussion

Consistent with previous reports, mice reliably discriminated PCP from saline, and this discrimination generalized to dizocilpine. The potency of PCP did not differ between the pre-inoculation and 3 week post-inoculation conditions, but increased approximately fivefold at 6 weeks and 9 weeks post-inoculation. This time course is similar to that reported for demonstration of other behavioral and neurochemical effects of LP-BM5 (Sei et al., 1992b; Ha et al., 1995; Sei et al., 1996; English et al., 1998).

It is important to consider several hypotheses to explain the results. One is that the animals simply lost the specificity of the drug discrimination. However, amphetamine given nine weeks post-inoculation did not produce PCP-like responding. Another possibility is that the data may represent a sensitization effect. Indeed, sensitization to the effects of PCP after subchronic administration is reported (Xu and Domino, 1994a; Xu and Domino, 1994b; Xu and Domino, 1997). However, the locomotor responses that have been used as measures of sensitization are likely mediated by serotonergic and dopaminergic effects of PCP (Gleason and Shannon, 1997; Gleason and Shannon, 1998). In addition, the reported sensitization to PCP occurred after only 4 days of dosing. In our experiment animals had undergone approximately nine months of training and testing before inoculation. Any sensitization would have occurred during the training period and responses were stable at the time of inoculation. Response rates when PCP was administered were stable over the course of the experiment, although saline responding rates decreased. This may indicate increased responsiveness to the locomotor effects of PCP. However, amphetamine did not substitute for PCP, even when the dose was high enough to disrupt behavior.

Changes outside the CNS may have been causal or mediating factors in the apparent increased potency of PCP in our experiment. One possibility is that the potency change was due to an LP-BM5-caused increase in blood–brain barrier permeability like that reported in AIDS (Power et al., 1993). However, the PCP molecule is small and highly lipophilic, and therefore easily traverses even the intact blood–brain barrier (Baldridge and Bessen, 1990). Thus, we consider this to be an unlikely explanation of our results. Another non-CNS mechanism that might influence the apparent potency of PCP is decreased production of hepatic cytochrome *P*-450, an enzyme important for metabolism and excretion of foreign compounds. However, although such a decrease has been reported after LP-BM5 infection, it did not occur until 12 weeks post-inoculation (Ansher et al., 1994). Thus, we conclude that a central mechanism of action, functional changes in NMDA receptors, is the likely cause of the increased potency.

The data we present do not exhaustively characterize the relationship between LP-BM5 infection, neurochemical changes, and PCP discrimination, but do suggest several hypotheses. One is that the relative affinity of NMDA receptors for antagonists (vs. agonists) increases after infection. A reduction in the relative affinity of the receptor for the naturally occurring agonist could decrease glutamatergic neurotransmission; if this decrease and PCP-induced Ca^{2+} blockade are additive, this might lower the threshold at which a 'PCP-like' state is achieved. This hypothesis is consistent with the finding that antagonist binding increases at the glycine and glutamate sites after LP-BM5 infection, whereas the potency of glycine to displace the antagonist 5,7 dichlorokynurenic acid decreases (English et al., unpublished data). The present data may be of interest in application to problems of drug abuse, since HIV infection is widespread in drug abusers (CDC, 1996). An analogous increase in drug sensitivity in AIDS cases could result in increased likelihood of overdose or enhanced prominence of NMDA receptor antagonist activity in the effects of drugs acting on multiple receptor systems. Further investigation of the LP-BM5 virus' effects on other drugs of abuse will help establish the generality and specificity of the results.

Supported by NIMH grant MH-53228 to I.A.P.

References

Ansher, S., Thompson, W., Watson, R., 1994. Alterations of hepatic drug metabolism in mice following infection with the murine retrovirus LP-BM5. Immunopharmacology 27, 215–223.

Baldridge, E.B., Bessen, H.A., 1990. Phencyclidine. Emergency Medical Clinics of North America 8, 541–550.

Balster, R.L., 1991. Discriminative stimulus properties of phencyclidine and other NMDA antagonists. NIDA Research Monograph 116, 163–180.

Balster, R.L., Moser, V.C., 1987. Pentobarbital discrimination in the mouse. Alcohol and Drug Research 7, 233–242.

Borlongan, C.V., Watanabe, S., 1995. A rapid assessment of stimulus properties of morphine. Life Sci. 57, 171–174.

Buller, R.M., Yetter, R.A., Fredrickson, T.N., Morse, H.C., 1987. Abrogation of resistance to severe mousepox in C57BL/6 mice infected with LP-BM5 murine leukemia viruses. J. Virol. 61, 383–387.

Butcher, S.P., Davis, S., Morris, R.G., 1990. A dose-related impairment of spatial learning by the NMDA receptor antagonist 2-amino-5-phosphonovalerate. Eur. Neuropsychopharmacol. 1, 15–20.

Butcher, S.P., Hamberger, A., Morris, R.G., 1991. Intracerebral distribution of DL-2-aminophosphopentanoic acid (AP5) and the dissociation of different types of learning. Exp. Brain Res. 83, 521–526.

CDC, 1996. HIV/AIDS Surveillance Report. Centers for Disease Control and Prevention, Atlanta.

Cotman, C.W., Monaghan, D.T., Ottersen, O.P., Storm-Mathisen, J., 1987. Anatomical organization of excitatory amino acid receptors and their pathways. TINS 10, 273–280.

Davis, S., Butcher, S.P., Morris, R.G., 1992. The NMDA receptor antagonist D-2-amino-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. J. Neurosci. 12, 21–34.

English, J.A., Hemphill, K.M., Paul, I.A., 1998. LP-BM5 infection impairs acquisition, but not performance, of active avoidance responding in C57Bl/6 mice. FASEB J. 12, 175–180.

Geter-Douglass, B., Witkin, J.M., 1997. Dizocilpine-like discriminative stimulus effects of competitive NMDA receptor antagonists in mice. Psychopharmacology (Berl) 133, 43–50.

Gleason, S.D., Shannon, H.E., 1997. Blockade of phencyclidine-induced hyperlocomotion by olanzapine, clozapine and serotonin receptor subtype selective antagonists in mice. Psychopharmacology (Berl) 129, 79–84.

Gleason, S.D., Shannon, H.E., 1998. Meta-chlorophenylpiperazine induced changes in locomotor activity are mediated by 5-HT1 as well as 5-HT2C receptors in mice. Eur. J. Pharmacol. 341, 135–138.

Ha, J.-H., Sei, Y., Basile, A.S., 1995. Striatal met-enkephalin and substance P levels are decreased in mice infected with the LP-BM5 murine leukemia virus. J. Neurochem. 64, 1896–1898.

Hartley, J.W., Fredrickson, T.N., Yetter, R.A., Makino, M., Morse, H.C., 1989. Retrovirus-induced murine acquired immunodeficiency syndrome: natural history of infection and differing susceptibility of inbred mouse strains. J. Virol. 63, 1223–1231.

Jolicoeur, P., 1991. Murine acquired immunodeficiency syndrome (MAIDS): an animal model to study AIDS pathogenesis. Vol. 5, pp. 2398–2405.

Klinken, S.P., Fredrickson, T.N., Hartley, J.W., Yetter, R.A., Morse, H.C., 1988. Evolution of B cell lineage lymphomas in mice with a retrovirus-induced immunodeficiency syndrome. MAIDS 140, 1123–1131.

Klinman, D.M., Morse, H.C., 1989. Characteristics of B cell proliferation and activation in murine AIDS. J. Immunol. 142, 1144–1149.

Lodge, D., Johnson, K.M., 1990. Noncompetitive excitatory amino acid receptor antagonists. Trends Pharmacol. Sci. 11, 81–86.

Martin, D., Lodge, D., 1988. Phencyclidine receptors and *N*-methyl-D-aspartate antagonism. Vol. 31, pp. 279–286.

Mosier, D.E., Yetter, R.A., Morse, H.C., 1989. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57/BL6 mice. J. Exp. Med. 161, 766–784.

Navia, B.A., Jordan, B.D., Price, R.W., 1986. The AIDS dementia complex: I. Clinical features. Ann. Neurol. 19, 517–524.

Power, C., Kong, P.A., Crawford, T.O., Wesselingh, S., Glass, J.D., McArthur, J.C., Trapp, B.D., 1993. Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood–brain barrier. Ann. Neurol. 34, 339–350.

Rees, D.C., Coggeshall, E., Balster, R.L., 1985. Inhaled toluene produces pentobarbital-like discriminative stimulus effects in mice. Life Sci. 37, 1319–1325.

Rees, D.C., Knisely, J.S., Balster, R.L., Jordan, S., 1987. Pentobarbital-like discriminative stimulus properties of halothane, 1,1,1-trichloroethane, isoamyl nitrite, flurothyl and oxazepam in mice. J. Pharmacol. Exp. Ther. 241, 507–515.

Sei, Y., Arora, P.K., Skolnick, P., Paul, I.A., 1992a. Spatial learning impairment in a murine model of AIDS. Vol. 6, pp. 3008–3013.

Sei, Y., Makino, M., Vitkovic, L., Chattopadhyay, S.K., Hartley, J.W., Arora, P.K., 1992b. Central nervous system infection in a murine retrovirus-induced immunodeficiency syndrome. J. Neuroimmunol. 37, 131–140.

Sei, Y., Paul, I.A., Saito, K., Layer, R.T., Hartley, J.W., Morse, H.C., Skolnick, P., Heyes, M.P., 1996. Quinolinic acid levels in a murine retrovirus-induced immunodeficiency syndrome. J. Neurochem. 66, 296–302.

Snoddy, A.M., Tessel, R.E., 1983. Nisoxetine and amphetamine share discriminative stimulu properties in mice. Pharmacol. Biochem. Behav. 19, 205–210.

Willetts, J., Balster, R.L., 1988. Phencyclidine-like discriminative stimulus properties of MK-801 in rats. Eur. J. Pharmacol. 146, 167–169.

Willets, J., Balster, R.L., Leander, J.D., 1990. The behavioral pharmacology of NMDA receptor antagonists. Trends Pharmacol. Sci. 11, 423–428.

Xu, X., Domino, E.F., 1994a. Asymmetric cross-sensitization to the locomotor stimulant effects of phencyclidine and MK-801. Neurochem. Int. 25, 155–159.

Xu, X., Domino, E.F., 1994b. Phencyclidine-induced behavioral sensitization. Pharmacol. Biochem. Behav. 47, 603–608.

Xu, X., Domino, E.F., 1997. Cross-sensitization between phencyclidine and (−) but not (+) pentazocine. Pharmacol. Biochem. Behav. 56, 205–210.