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Pharmacology, Biochemistry and Behavior 79 (2004) 219–228
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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# Effects of abused inhalants and GABA-positive modulators in dizocilpine discriminating inbred mice☆

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Received 28 February 2004; received in revised form 4 June 2004; accepted 15 July 2004
Available online 29 September 2004

**Abstract**

There is in vitro evidence that some of the effects of abused volatile solvents may be produced by actions at the NMDA receptor. In addition, some solvents produce phencyclidine-like discriminative stimulus effects. The major goal of the present study was to further compare abused solvents to NMDA antagonists by testing them in two strains of mice trained to discriminate 0.17 mg/kg of the very selective uncompetitive NMDA antagonist, dizocilpine, from saline and contrast those results with several $GABA_A$-positive modulators, PCP and ethanol. The results indicated that the discriminative stimulus produced by 0.17 mg/kg dizocilpine was highly specific in both mouse strains. PCP produced 91% dizocilpine-lever responding in C57BL/6J mice, but only 56% dizocilpine-lever responding in DBA/2J mice. Pentobarbital, midazolam and ethanol produced at least some overlap in discriminative stimulus effects with dizocilpine in one or both mouse strains. In contrast, toluene, 1,1,1-trichloroethane (TCE), xylene and methoxyflurane produced saline-appropriate responding almost exclusively. These data indicate that, at least under the specific conditions tested, abused volatile solvents do not have substantial dizocilpine-like discriminative stimulus effects in either C57BL/6J or DBA/2J mice, providing little support that NMDA antagonism plays a central role in the production of this abuse-related effect.


*Keywords:* Drug discrimination; C57BL6/J; DBA/2J; Dizocilpine; Solvent; Inbred mice

## 1. Introduction

The abuse of volatile solvents is a serious social and medical problem both in the United States and worldwide. The prevalence of volatile solvent abuse is highest among children. The most recent data from the 2002 Monitoring the Future Study Survey found that 15.2% of 8th graders reported inhaling volatile solvents at least once (Johnston et al., 2003). Despite the problem, the neural bases for the abuse-related behavioral effects of volatile solvents are poorly understood (Balster, 1998). Of particular importance are the behavioral and neurochemical effects of these compounds following acute exposures at the high concentrations which they are typically abused.

Several behavioral studies have examined the acute intoxicating and neurochemical effects of toluene and 1,1,1-trichloroethane (TCE) (Bowen and Balster, 1996; Evans and Balster, 1991, 1993; Wiley et al., 2002). In general, toluene and TCE share many common behavioral and neurochemical effects with $GABA_A$-positive modulators like benzodiazepines, barbiturates, volatile anesthetics and ethanol (Balster, 1998; Beckstead et al., 2000). Toluene and TCE also share some behavioral effects with drugs that act as uncompetitive antagonists of the NMDA subtype of glutamate receptors (Balster, 1998) as well as acting as NMDA antagonists in vitro (Cruz et al., 1998, 2000).

Of particular importance to the abuse-related effects of volatile solvents are studies on their discriminative stimulus effects. In drug discrimination studies, toluene

☆ Supported by NIDA Grants DA-03221, DA-01442 and NIAAA grant AA-13665.
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doi:10.1016/j.pbb.2004.07.009

and TCE produce partial substitution in mice trained to discriminate diazepam from saline (Bowen et al., 1999) and ethanol from saline (Balster et al., 1997; Rees et al., 1987). TCE and toluene also produce partial substitution for the uncompetitive NMDA antagonist, phencyclidine, in mice (Bowen et al., 1999). These studies suggest that, much like ethanol, the discriminative stimulus of volatile solvents may be a mixed cue based on both NMDA antagonist and $GABA_A$ agonist activity (Grant, 1994). However, previous research comparing the discriminative stimulus properties of selective positive $GABA_A$ modulators and NMDA antagonists has shown that, under some conditions, drugs from these two classes show partial cross-substitution for one another (Mansbach and Balster, 1991; McMillan and Wessinger, 1989; Snodgrass and McMillan, 1991; Willetts and Balster, 1989). Thus, the phencyclidine-like discriminative stimulus effects of abused solvents may be due to discriminative stimulus overlap between NMDA antagonists and $GABA_A$ agonists rather than selective neurochemical actions at the NMDA receptor.

In the present study, we sought to more completely assess the NMDA antagonist-like effects of volatile solvents and compare and contrast those effects to midazolam and pentobarbital, which are known to act through the $GABA_A$ receptor complex. In a previous experiment from this laboratory, the phencyclidine-like effects of volatile solvents were assessed in outbred Swiss–Webster mice (Bowen et al., 1999). Although the behavioral effects of phencyclidine are almost certainly largely mediated by NMDA receptors, PCP does have other actions, including activity at dopamine receptors (Crosby et al., 2002; Maurice et al., 1991). Therefore, in the present study we chose to train a discrimination based on the prototypic selective uncompetitive NMDA antagonist, dizocilpine. Dizocilpine, unlike PCP, is thought to be devoid of dopaminergic effects and might provide a clearer indicator of the uncompetitive NMDA antagonist-like effects of volatile solvents.

We trained the dizocilpine versus saline discrimination in both C57BL/6J and DBA/2J mice. These two strains are perhaps the best characterized and compared of all inbred mouse strains. In particular, C57BL/6J and DBA/2J mice have vastly different behavioral responses to ethanol which are too numerous to detail (Phillips, 1997). Given the common behavioral effects of volatile solvents and ethanol it was thought that comparing these strains might prove useful insights into the similarities and differences between volatile solvents and ethanol (Balster, 1998). C57BL/6J and DBA/2J mice may also have utility for examining differences in NMDA-receptor mediated effects. For instance, C57BL/6J and DBA/2J mice are the basis for BXD/RI strains used to detect several potential quantitative trait loci for phencyclidine-induced locomotion (Alexander et al., 1996). Hippocampal pyramidal cells from DBA/2J mice are more sensitive to NMDA-induced spontaneous discharges than cells from C57BL/6J mice (Wang and Chow, 1995) and DBA/2J mice are more prone to experimentally induced audiogenic seizures than C57BL/6J mice, an effect that is believed to be due at least in part to NMDA receptor activation (Engstrom and Woodbury, 1988). C57BL/6J and DBA/2J mice also differ quite markedly in neurophysiology as well as in their responses to other drugs such as opioids, psychomotor stimulants and $GABA_A$ agonists (Cunningham et al., 1999; Finn et al., 1997; Jamensky and Gianoulakis, 1999; Kiianmaa et al., 1983; Ng et al., 1996; Risinger et al., 1998).

## 2. Methods

### 2.1. Subjects

Twelve C57BL/6J and eleven DBA/2J mice (Jackson Laboratory, Bar Harbor, ME) served as subjects. The mice were 9–10 weeks old at the start of discrimination training. The mice were individually housed on a 12 h light/dark cycle (lights on 7 AM) and allowed to acclimate to the laboratory for a period of 1 week prior to the start of training. The animals were fed sufficient rodent chow (Harlan, Teklad, Madison, WI) following sessions and on weekends to maintain stable body weights of between 25 and 31 g for the duration of the study.

### 2.2. Drugs

Dizocilpine maleate (MK-801) and pentobarbital sodium were purchased from Sigma (St. Louis, MO). Midazolam hydrochloride was obtained in an injectable formulation (VERSED, Roche Pharmaceuticals, Nutley, NJ) from Virginia Commonwealth University Medical Center central pharmacy. Ethanol was obtained from Virginia Commonwealth University central stores. Phencyclidine HCl was provided by the National Institute on Drug Abuse. Toluene and 1,1,1-trichloroethane were purchased from Aldrich Chemicals (Milwaukee, WI). Racemic xylene was obtained from Fisher Chemical Division (Fair Lawn, NJ). Methoxyflurane (Metofane) was purchased from Pitman-Moore (Mundelein, IL).

Dizocilpine (0.03, 0.1, 0.17, 0.3, 0.56 mg/kg), phencyclidine (1, 2, 4, 8, 12 mg/kg), pentobarbital (3, 10, 17, 30 mg/kg), midazolam (0.3, 1, 3, 10, 30, 56 mg/kg) and ethanol (100, 300, 100, 1500, 2000, 3000 mg/kg) were administered, i.p., 10 min prior to the start of the test session. All drugs given via injection were diluted in sterile saline to maintain a constant injection volume of 10 ml/kg. Doses were based on the weights of the salts. For volatile vapors, mice were exposed for 10 min immediately prior to the start of the discrimination test session to TCE (4000, 8000, 16,000 ppm), toluene (1000, 2000, 4000, 6000 ppm), xylene (1000, 2000, 4000, 6000 ppm) and methoxyflurane (500, 1000, 2000, 4000 ppm).

### 2.3. Apparatus

Drug discrimination sessions were conducted in standard mouse operant conditioning chambers (Med-associates, St. Albans, VT). Each chamber was equipped with two levers on the front wall of the operant chamber. Above each lever was a yellow LED stimulus lamp. Equidistant between the levers was a recessed receptacle into which a 0.1-ml liquid dipper cup could be elevated via an electrically operated dipper mechanism. A single 5 W houselight was located at the top center of the chamber rear wall. The operant conditioning chambers were individually housed in sound-attenuating and ventilated cubicles. Drug discrimination schedule conditions and data recording were accomplished using a Med-associates interface and Med-PC version 4 control software running on an PC-compatible computer (Med-Associates). The milk solution reinforcer consisted of 25% sugar, 25% nonfat powdered milk and 50% tap water (by volume).

The static vapor chambers used to expose the mice to solvent and anesthetic vapors prior to drug discrimination testing have been described previously (Bowen and Balster, 1996). Briefly, each chamber consisted of a 29-l cylindrical glass bell jar with a clear acrylic lid and attached fan motor. A foam rubber gasket was fixed to the rim of the bell jar to insure a tight seal. A drive shaft with sealed bearings extended through the lid into the chamber where it was connected to a plastic fan blade. Directly below the fan blade was a suspended wire mesh platform to which a filter paper disk was attached. After a mouse was placed into the chamber and the lid sealed, a measured volume of a volatile solvent or anesthetic calculated to produce the desired chamber concentration (Nelson, 1971) was drawn into a glass syringe and injected via a stoppered port in the chamber lid onto the filter paper. The fan was then activated, volatilizing and distributing the vapor throughout the chamber. Exposure vapor concentrations were verified using a single wavelength monitoring infrared spectrometer (Miran 1A, Foxboro Analytical, North Haven, CT). Control testing verified that chamber concentration reached equilibrium within 1 min for all vapors tested and did not vary measurably for the duration of the 10-min exposure.

### 2.4. Discrimination training

After the animals were adapted to the laboratory, daily (Monday–Friday) 15-min training sessions were initiated. The mice were first trained to press one lever under a fixed ratio 1 response (FR-1) schedule. Upon completion of the FR requirement, a 0.1 ml liquid dipper cup was elevated into the dipper receptacle for 5 s Responses occurring while the dipper was elevated did not count toward completion of the next ratio requirement. Responding on the inactive lever reset the FR requirement on the correct lever. The animals were then trained to respond on the opposite lever under the FR-1 schedule. During experimental sessions, both stimulus lights and the house light were illuminated for the duration of the session. Drug discrimination training began when an animal responded reliably on both levers for several sessions. During each discrimination training session, one of the two levers was designated as correct. The correct lever was determined by whether the subject received an i.p. injection of either 0.17 mg/kg dizocilpine or saline. Completion of the FR requirement on the correct lever resulted in 5 s of dipper availability. The lever corresponding to dizocilpine and saline pretreatments remained fixed for the duration of the study for a given animal but was counterbalanced across mice. Dizocilpine and saline were injected on a double alternation schedule (i.e. two dizocilpine days followed by two saline days). Responses emitted on the incorrect lever were recorded and reset the FR requirement on the correct lever. Over the course of a number of sessions, the response requirement was increased to FR-12. These training conditions were in effect for the remainder of the study. Animals were determined to have acquired the dizocilpine and saline discrimination when the first FR was completed on the correct lever, prior to the completion of a FR on the incorrect lever, in 8 out of 10 consecutive sessions. Additionally, the mice were required to emit greater than 80% of responses on the correct lever during all 10 of these sessions.

### 2.5. Substitution test procedure

Following acquisition of the 0.17 mg/kg i.p. dizocilpine and saline discrimination, substitution tests were conducted on Tuesday and Friday, providing that the mice continued to exhibit accurate stimulus control on the Monday, Wednesday and Thursday training sessions. Test sessions were suspended if an animal did not emit the first FR on the correct lever and produce greater than 80% correct-lever responding during all training sessions since the last test session. If a mouse did not meet the criteria for testing, it continued to receive additional dizocilpine and saline training sessions until the correct first FR, as well as greater than 80% correct-lever responding, was emitted for a minimum of three consecutive training sessions. Between substitution tests, the double alternation sequence of dizocilpine and saline training sessions was continued. Substitution test sessions with drugs given by injection commenced after a 10-min pretreatment interval. Substitution test sessions using volatile solvents and anesthetics were preceded by a saline injection and 10-min exposure to a single concentration of vapor. The animal was then immediately removed from the exposure chambers and placed into the operant chamber for a 2-min drug discrimination test session. On test days, both levers were active and completion of a FR requirement on either lever resulted in dipper presentation. Test drugs or vapor

concentrations were administered in an ascending order until the mean response rate of the group was suppressed to less than 50% of the group saline control value or other considerations precluded testing higher doses or concentrations. Each drug dose or concentration was administered once without regard for the prior days training condition (dizocilpine or saline). Prior to each dose–effect curve, control tests with 0.17 mg/kg dizocilpine and saline were conducted. Mice from each strain were assigned to a test drug after the completion of the dizocilpine dose–effect curve. Once assigned to a drug dose–effect curve, a mouse received every test dose or concentration of a given compound in ascending order. Not all animals received all drugs in order to maximize the number of compounds tested.

### 2.6. Data analysis

Dizocilpine and saline lever responses and dipper presentations were recorded for each animal. Group means (±SEM) were calculated for both percentage dizocilpine-lever responding and response rate at each drug dose. During drug discrimination tests, the percentage of responses emitted on the dizocilpine-appropriate lever during the entire test session was used as a measure of the ability of a test drug to substitute for the 0.17 mg/kg dizocilpine training dose. To increase the reliability of the lever-selection data, any drug dose or vapor concentration that suppressed rates to the extent that at least one dipper presentation was not earned by a mouse resulted in the exclusion of that mouse's data point from the group lever-selection analysis, although that animal's data was included in the response rate determination. The saline control response rate for an individual animal was defined as total responses/session on both levers during the saline control session that preceded each dose–effect curve. Response rate for each test drug dose was converted to a percentage of the saline control rate for individual animals. Group mean percentage of saline control rate (±SEM) for each test dose was calculated from the individual animal data. A criteria of 80% or greater dizocilpine-appropriate responding was selected to indicate full substitution for the 0.17 mg/kg dizocilpine training dose. Mean dizocilpine-lever responding between 20% and 79% was defined as partial substitution. Mean dizocilpine-lever responding of less than 20% was considered to be evidence of no substitution for dizocilpine. $ED_{50}$ values (and 95% confidence limits) for dizocilpine-lever selection and response rate suppression were calculated based on the linear portion of each mean dose–effect curve. Calculations were performed using a Microsoft Excel spreadsheet based on SAS Pharm/PCS version 4 (Tallarida and Murray, 1986). $ED_{50}$ values between strains were deemed significantly different when 95% confidence levels for each strain did not overlap.

## 3. Results

### 3.1. Acquisition and dizocilpine substitution

All 12 C57BL/6J and 11 DBA/2J mice acquired the 0.17 mg/kg dizocilpine and saline discrimination. The number of days to acquisition was not significantly different between C57BL/6J and DBA/2J mice (C57BL/6J=48.5±4.2 days, DBA/2J=44±3.3 days). One C57BL/6J and three DBA/2J mice died after acquisition, but before completing the dizocilpine dose–effect curve. As a consequence, 11 C57BL/6J and 9 DBA/2J mice were used to determine the dizocilpine dose–effect curve. The control test with 0.17 mg/kg dizocilpine resulted in greater than 80% dizocilpine-lever selection and control test with saline resulted less than 10% dizocilpine-lever responding in both strains. The 0.17 and 0.3 mg/kg test doses of dizocilpine fully substituted for the training dose in both C57BL/6J and DBA/2J mice. (Fig. 1, upper panel). There was no significant difference in the $ED_{50}$ value for substitution between C57BL/6J mice (0.08 mg/kg [CL: 0.06–0.12 mg/kg]) and DBA/2J mice (0.11 mg/kg [CL: 0.08–0.15 mg/kg]). Operant response rates in both the dizocilpine and saline control test sessions were comparable between strains. Specifically, saline pretreatment resulted in response rates of 22±2.3 and 19.5±6.5 responses/min in C57BL/6J and DBA/2J mice, respectively. The 0.17 mg/kg dizocilpine control session produced response rates of 21.6±4.2 responses/min in C57BL/6J mice and 19.8±6.6 responses/min in DBA/2J mice. Dizocilpine had a biphasic effect on response rates in both strains of mice. The 0.03 and 0.1 mg/kg doses produced modest rate increasing effects, whereas the highest, 0.56 mg/kg dose of dizocilpine almost completely suppressed response rates in both C57BL/6J and DBA/2J mice (Fig. 1, lower panel). There was no significant difference in the $ED_{50}$ value for dizocilpine rate suppression between C57BL/6J mice (0.38 mg/kg [CL: 0.32–0.46 mg/kg]) and DBA/2J mice (0.36 mg/kg [CL: 0.31–0.43 mg/kg]).

### 3.2. Test drug substitution

Nine C57BL/6J and six DBA/2J mice were used to determine phencyclidine substitution for dizocilpine. Phencyclidine dose-dependently substituted for dizocilpine in C57BL/6J mice (Fig. 2, upper panel). In C57BL/6J mice, a maximum of 91% dizocilpine-lever selection was engendered by the 8 mg/kg phencyclidine dose which also produced pronounced rate-suppressing effects (Fig. 2, lower panel). In contrast, phencyclidine produced a maximum of 56% dizocilpine-lever responding in DBA/2J mice (Fig. 2, upper panel), but only three of six DBA/2J mice emitted at least one complete FR value at this dose (Fig. 2, lower panel). There was no significant difference between $ED_{50}$'s for phencyclidine substitution in C57BL/6J (3.4 mg/kg [CL: 2.8–4.3 mg/kg]) and DBA/2J mice (6.4 mg/kg [CL: 3.6–11.1 mg/kg]). Although DBA/2J mice were slightly more

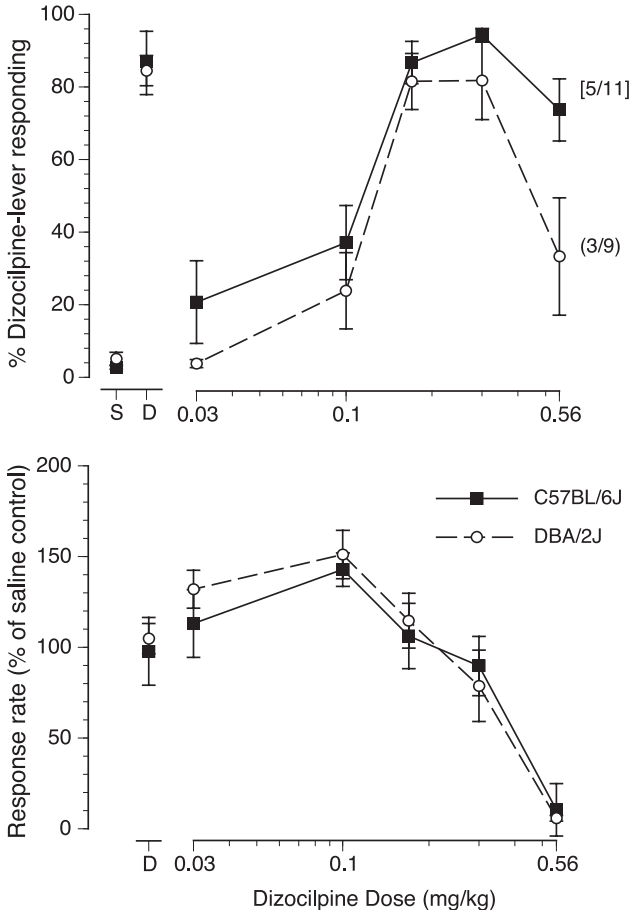


Fig. 1. Dose–effect curves for dizocilpine substitution in eleven C57BL/6J (■) and nine DBA/2J (○) mice trained to discriminate 0.17 mg/kg i.p. dizocilpine from saline. Points above S and D represent the results of saline and 0.17 mg/kg i.p. dizocilpine control sessions. Mean (±SEM) percentage of dizocilpine-lever responding is shown in the top panel. Mean (±SEM) response rate expressed as a percentage of saline control rate is shown in the bottom panel. Numbers in square brackets denote that only the indicated number of C57BL/6J mice out of the entire group completed at least one FR value at that dose. Values in parentheses indicate the same information for DBA/2J mice.

sensitive to the rate-suppressing effects of phencyclidine, there was no significant difference in $ED_{50}$'s for rate suppression between C57BL/6J (7.4 mg/kg [CL: 5.6–9.9 g/kg]) and DBA/2J mice (5.1 mg/kg [CL: 4.4–5.9 mg/kg]).

Fig. 3 (upper panel) shows pentobarbital substitution for dizocilpine in C57BL/6J ($n$=9) and DBA/2J ($n$=6) mice. Pentobarbital produced dose-dependent substitution for dizocilpine only in DBA/2J mice, producing a maximum of 50% dizocilpine-lever responding at the 30 mg/kg dose. However, only three of the six DBA/2J mice tested at this dose of pentobarbital emitted at least one complete FR. In contrast, pentobarbital producing a maximum of 15% dizocilpine-lever responding at the highest dose in C57BL/6J mice. The lower panel of Fig. 3 shows the effect of pentobarbital on rates of operant responding in C57BL/6J and DBA/2J mice. Only the 30 mg/kg pentobarbital dose had any response rate-decreasing effects in either strain. The $ED_{50}$ for response rate suppression by pentobarbital was not significantly different between C57BL/6J (29.9 mg/kg [CL: 22.9–39.0 mg/kg]) and DBA/2J mice (23.7 mg/kg [CL: 21.2–26.5 mg/kg]).

Midazolam produced dose-dependent increases in dizocilpine-lever section in both C57BL/6J ($n$=8) and DBA/2J ($n$=6) mice (Fig. 4, upper panel). A maximum of 67% dizocilpine-lever selection was produced by the 56 mg/kg midazolam dose which also completely suppressed operant responding in five of the eight C57BL/6J mice tested. The $ED_{50}$ for midazolam substitution in C57BL/6J mice was 17.3 mg/kg [CL: 8.9–33.7 mg/kg]. In DBA/2J mice, maximal dizocilpine-lever selection of 30% was engendered by the 10 mg/kg midazolam dose (Fig. 4, upper panel). Lower doses of midazolam increased, whereas higher doses decreased response rates in both C57BL/6J and DBA/2J mice (Fig. 4, lower panel). The $ED_{50}$ for response rate

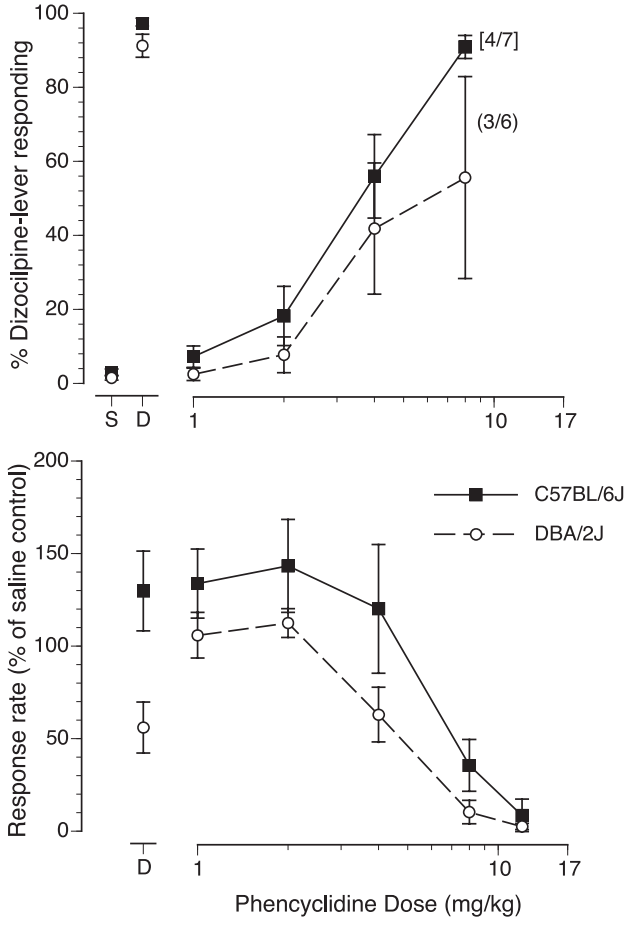


Fig. 2. Dose–effect curves for phencyclidine substitution in nine C57BL/6J (■) and six DBA/2J (○) mice trained to discriminate 0.17 mg/kg i.p. dizocilpine from saline. Points above S and D represent the results of saline and 0.17 mg/kg i.p. dizocilpine control sessions. Mean (±SEM) percentage of dizocilpine-lever responding is shown in the top panel. Mean (±SEM) response rate expressed as a percentage of saline control rate is shown in the bottom panel. Numbers in square brackets denote that only the indicated number of C57BL/6J mice out of the entire group completed at least one FR value at that dose. Values in parentheses indicate the same information for DBA/2J mice.

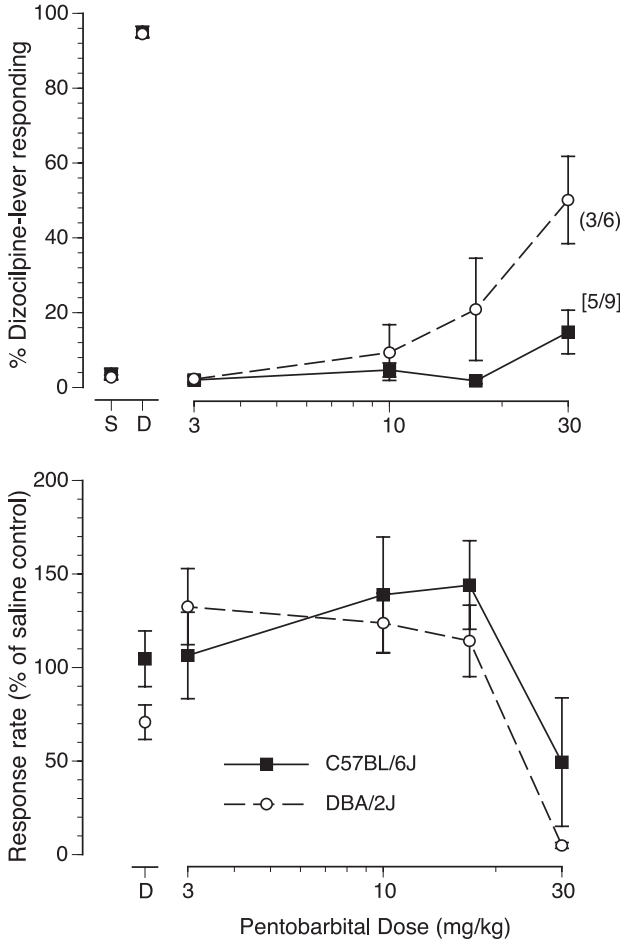


Fig. 3. Dose–effect curves for pentobarbital substitution in nine C57BL/6J (■) and six DBA/2J (○) mice trained to discriminate 0.17 mg/kg i.p. dizocilpine from saline. Points above S and D represent the results of saline and 0.17 mg/kg i.p. dizocilpine control sessions. Mean (±SEM) percentage of dizocilpine-lever responding is shown in the top panel. Mean (±SEM) response rate expressed as a percentage of saline control rate is shown in the bottom panel. Numbers in square brackets denote that that only the indicated number of C57BL/6J mice out of the entire group completed at least one FR value at that dose. Values in parentheses indicate the same information for DBA/2J mice.

suppression by midazolam in DBA/2J mice (3.1 mg/kg [CL: 1.5–6.1 mg/kg]) was significantly lower than that in C57BL/6J mice (22.3 mg/kg [CL: 13.8–36.0 mg/kg]).

Table 1 shows the results of substitution testing with ethanol, TCE, toluene, xylene and methoxyflurane in C57BL/6J and DBA/2J mice. Maximal substitution of 42% dizocilpine-lever selection in C57BL/6J and 23% dizocilpine-lever selection in DBA/2J mice was produced at the 1500 mg/kg ethanol dose. Ethanol dose-dependently suppressed operant responding in both strains to an equal degree with the 3000 mg/kg dose completely suppressing response rates in both C57BL/6J and DBA/2J mice. The $ED_{50}$ for response rate suppression by ethanol was 2000 mg/kg [CL: 1700–2300 mg/kg] in C57BL/6J and 2200 mg/kg [CL: 2000–2600 mg/kg] in DBA/2J mice. None of the concentrations of TCE produced any greater than 8% dizocilpine-lever selection in DBA/2J ($n$=6) mice. In C57BL/6J ($n$=8) mice, the 16,000 ppm TCE concentration produced a maximum of 32% dizocilpine-lever selection. Of the six C57BL/6J mice that completed at least one FR value at 16,000 ppm TCE, three animals predominately responded on the saline-appropriate lever, two emitted somewhat more responses on the saline lever than the dizocilpine lever and only one C57BL/6J mouse responded exclusively on the dizocilpine-appropriate lever. The 4000 and 8000 ppm TCE concentrations had little effect on response rates in C57BL/6J mice but 16,000 ppm TCE suppressed rates to 48% of saline control levels. TCE produced concentration-dependent reductions in operant responding in DBA/2J mice with 8000 and 16,000 ppm TCE concentrations resulting in 49% and 75% reductions, respectively, in operant responding compared to the saline+air control session. The $EC_{50}$ for suppression of operant responding in DBA/2J mice was

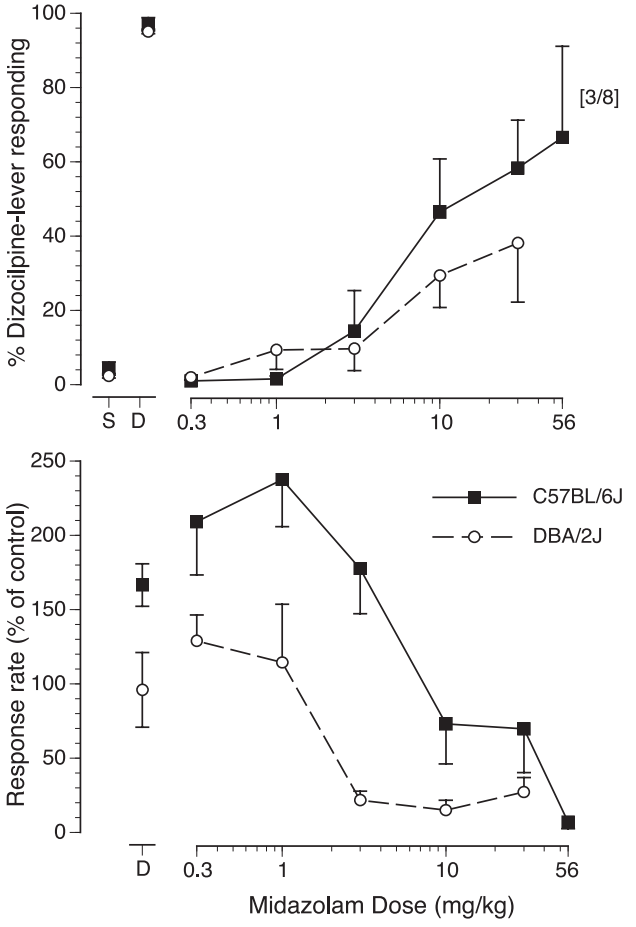


Fig. 4. Dose–effect curves for midazolam substitution in eight C57BL/6J (■) and six DBA/2J (○) mice trained to discriminate 0.17 mg/kg i.p. dizocilpine from saline. Points above S and D represent the results of saline and 0.17 mg/kg i.p. dizocilpine control sessions. Mean (±SEM) percentage of dizocilpine-lever responding is shown in the top panel. Mean (±SEM) response rate expressed as a percentage of saline control rate is shown in the bottom panel. Numbers in square brackets denote that that only the indicated number of C57BL/6J mice out of the entire group completed at least one FR value at that dose. Values in parentheses indicate the same information for DBA/2J mice.

Table 1
Percentage of dizocilpine-lever selection and operant response rates produced by ethanol, 1,1,1 trichloroethane (TCE), toluene, xylene and methoxyflurane in C57BL/6J and DBA/2J mice

| Drug | Ethanol dose or vapor concentration | Dizocilpine-lever responding (%) (±SEM) | | Response rate-control (%) (±SEM) | |
|---|---|---|---|---|---|
| | | C57BL/6J | DBA/2J | C57BL/6J | DBA/2J |
| Ethanol | saline | 1.9 (0.7) | 3.6 (0.9) | – | – |
| | dizocilpine | 87.6 (11.0) | 90.2 (3.3) | 109.7 (0.7) | 84.5 (13.7) |
| | 100 mg/kg | 0.9 (0.2) | 2.8 (0.8) | 97.8 (3.6) | 86.3 (12.0) |
| | 300 mg/kg | 1.4 (0.9) | 4.5 (1.3) | 118.2 (18.2) | 105.4 (14.5) |
| | 1000 mg/kg | 8.3 (3.4) | 3.0 (1.5) | 109.1 (11.8) | 126.2 (14.3) |
| | 1500 mg/kg | 10.8 (4.4) | 6.4 (3.1) | 97.3 (19.8) | 116.1 (21.0) |
| | 2000 mg/kg | 42.4 (18.3)[a] 6/9 | 23.3 (17.8) | 44.3 (16.4) | 70.7 (16.6) |
| | 3000 mg/kg | – | – | 8.3 (5.3) | 1.3 (1.3) |
| TCE | air+saline | 1.6 (0.9) | 1.2 (0.3) | – | – |
| | air+dizocilpine | 98.2 (0.7) | 93.2 (4.7) | 136.2 (30.3) | 116.2 (72.6) |
| | 4000 ppm | 9.4 (5.6) | 4.8 (2.0) | 93.4 (26.4) | 120.6 (48.5) |
| | 8000 ppm | 7.8 (7.1) | 7.7 (4.6) | 89.7 (32.5) | 51.1 (11.6) |
| | 16,000 ppm | 32.9 (15.6)[a] 6/8 | 2.3 (1.4)[a] 4/6 | 46.5 (18.9) | 24.6 (8.2) |
| Toluene | air+saline | 3.6 (2.4) | 7.6 (2.9) | – | – |
| | air+dizocilpine | 96.6 (2.2) | 92.3 (2.7) | 168.2 (38.0) | 57.3 (16.0) |
| | 1000 ppm | 5.2 (2.2) | 2.7 (0.6) | 75.1 (20.9) | 113.7 (17.1) |
| | 2000 ppm | 9.4 (3.8) | 4.0 (1.3)[a] 5/6 | 67.1 (12.5) | 73.5 (22.8) |
| | 4000 ppm | 19.1 (10.0) | 17.4 (9.6)[a] 4/6 | 57.3 (12.8) | 43.2 (12.8) |
| | 6000 ppm | 18.5 (10.6)[a] 6/8 | – | 40.5 (14.7) | 3.9 (2.0) |
| Xylene | air+saline | 2.5 (1.1) | 3.7 (1.5) | – | – |
| | air+dizocilpine | 98.3 (1.0) | 92.1 (2.7) | 183.8 (45.0) | 56.2 (10.0) |
| | 1000 ppm | 2.6 (0.8) | 2.7 (1.6) | 113.6 (24.8) | 81.4 (11.6) |
| | 2000 ppm | 3.3 (1.6)[a] 7/9 | 8.3 (5.0) | 94.0 (22.5) | 80.2 (11.3) |
| | 4000 ppm | 0.0 (0.0)[a] 3/9 | 10.8 (5.0) | 55.6 (25.4) | 91.6 (10.8) |
| | 6000 ppm | – | 8.0 (2.8)[a] 3/6 | 9.8 (6.2) | 85.6 (4.2) |
| Methoxyflurane | air+saline | 1.2 (1.2) | 0.8 (0.5) | – | – |
| | air+dizocilpine | 91.6 (5.4) | 89.8 (3.6) | 107.5 (27.3) | 61.3 (13.7) |
| | 500 ppm | 1.3 (1.3) | 4.0 (4.0) | 106.6 (26.9) | 108.8 (34.6) |
| | 1000 ppm | 15.4 (14.4) | 10.3 (3.0) | 122.6 (23.0) | 89.5 (18.6) |
| | 2000 ppm | 1.8 (1.0)[a] 4/5 | 7.3 (5.0)[a] 3/4 | 103.7 (33.8) | 50.3 (11.7) |
| | 4000 ppm | – | – | 15.3 (8.3) | 9.4 (2.5) |

[a] Indicate that data are based upon only those animals that completed at least one complete FR at that dose.

9997 ppm [CL: 6026–16583 ppm]. The $EC_{50}$ for rate suppression in C57BL/6J mice could not be determined accurately due to individual variability.

No concentration of toluene produced more than 19% dizocilpine-lever selection in either C57BL/6J ($n$=8) or DBA/2J ($n$=6) mice. However, toluene suppressed operant responding in a concentration-dependent manner in both strains (Table 1). The $EC_{50}$'s for suppression of operant responding were not significantly different between C57BL/6J (4576 ppm [CL: 1937–10,806 ppm]) and DBA/2J (3095 ppm [CL: 2289–4184 ppm]) mice The highest, 6000 ppm, toluene concentration almost completely suppressed operant responding in DBA/2J mice, but only suppressed responding to 41% of saline+air control levels in C57BL/6J mice.

Xylene, like TCE and toluene, failed to produce more than 11% dizocilpine-lever selection in either C57BL/6J ($n$=9) or DBA/2J ($n$=6) mice. Unlike TCE or toluene, xylene had a much more potent response rate-suppressing effect in C57BL/6J than DBA/2J mice. In C57BL/6J mice, xylene produced concentration-dependent response rate-suppressing effects with the highest, 6000 ppm, concentration suppressing operant responding by 90%. The $EC_{50}$ for response rate suppression in C57BL/6J mice was 3646 ppm [CL: 2490–5337 ppm]). The $EC_{50}$ for response rate suppression could not be determined in DBA/2J mice as xylene had only modest non-concentration-dependent effects on operant responding across the same concentration range.

As with the volatile solvents, methoxyflurane at concentrations of 500–4000 ppm produced no greater than 15% dizocilpine-lever selection in either C57BL/6J ($n$=5) or DBA/2J ($n$=4) mice, despite concentration-dependent suppression of operant responding in both strains. Although there was a trend toward more potent rate-suppressing effects in DBA/2J mice, there was no significant difference in the $EC_{50}$'s for rate suppression between C57BL/6J (2967 ppm [CL: 1997–4408 ppm]) and DBA/2J (1904 ppm [CL: 1244–2915 ppm]) mice.

## 4. Discussion

Both strains learned to discriminate dizocilpine from saline and there was no significant difference between

strains in discrimination learning rates. The rate of acquisition of a drug versus saline discrimination has been shown to be positively correlated with drug dose (De Vry and Slangen, 1986; Stolerman et al., 1999; York, 1978b). This would suggest that the discriminative stimulus salience of 0.17 mg/kg dizocilpine is comparable in C57BL/6J and DBA/2J mice. The present results are similar to a study showing that C57BL/6J and DBA/2J mice do not differ in their rate of learning a nicotine versus saline discrimination (Stolerman et al., 1999). However, in another experiment, DBA/2J mice were reported to learn a 1.5 g/kg ethanol discrimination more quickly than C57BL/6J mice (Shelton and Grant, 2002). Taken together, these findings suggest that drug discrimination learning in C57BL/6J and DBA/2J mice is dependent upon the specific training drug and not simply a difference between strains in the ability to learn a complex operant procedure.

In both C57BL/6J and DBA/2J mice, the discriminative stimulus effects of dizocilpine were highly selective. Only phencyclidine produced full substitution for dizocilpine, and that was at operant rate-suppressing doses and only in C57BL/6J mice. As both phencyclidine and dizocilpine are uncompetitive NMDA antagonists, the lack of complete substitution is somewhat surprising. However, at least two other studies, one in monkeys and the other in mice, have reported that phencyclidine does not always fully substitute in dizocilpine-trained animals (France et al., 1991; Geter-Douglass and Witkin, 1997). These results suggest that phencyclidine produces rate-suppressing behavioral effects that may impair it's ability to fully substitute for dizocilpine.

Pentobarbital produced 15% dizocilpine-lever responding in C57BL/6J mice and 51% dizocilpine-lever responding in DBA/2J mice. These findings, especially those in C57BL/6J mice, are somewhat at odds with the results of other studies that have found that pentobarbital produces no substitution for dizocilpine (Butelman et al., 1991; Witkin et al., 1997). Midazolam produced a maximum of 59% and 39% dizocilpine-lever selection in C57BL/6J and DBA/2J mice, respectively. To our knowledge, midazolam has not been tested in mice trained in a two-choice, dizocilpine versus saline discrimination. However, in Swiss–Webster mice trained to discriminate dizocilpine from saline in a T-maze procedure, diazepam exhibited a maximum of 60% substitution (Geter-Douglass and Witkin, 1997), almost identical to the 59% substitution generated in C57BL/6J mice in the present study. In the case of both pentobarbital and midazolam, partial substitution for dizocilpine was accompanied by pronounced rate-suppressing effects. While it is likely that pentobarbital and midazolam share some similarity in discriminative stimulus effects with dizocilpine, it is possible that these results were simply due to disruptions in the discrimination. This latter interpretation is unlikely for several reasons. Firstly, lever-selection data from the animals who did not earn at least one reinforcer were omitted from the group curves. Secondly, the rate-suppressing and discriminative stimulus effects of NMDA antagonists have been shown to be separable from one another and may be controlled by different determinants (Beardsley et al., 1987; Shelton and Grant, 2002). Thirdly, xylene, and to a lesser extent toluene, produced pronounced rate-suppressing effects, yet did not substitute for dizocilpine. Lastly, few of the animals at the high test doses of midazolam and pentobarbital divided their responding equally between the two levers, instead responding predominantly on only one lever.

Positive $GABA_A$ modulators and NMDA antagonists consistently substitute for ethanol (Grant, 1994; Grant et al., 1991; Sanger, 1993; Shelton and Balster, 1994). Conversely, ethanol generally only partially substitutes or does not substitute at all in animals trained to discriminate these classes of drugs from saline (Balster et al., 1992; Butelman et al., 1993; York, 1978a). In the present study, ethanol produced only 23% dizocilpine-lever selection in DBA/2J mice and 42% dizocilpine-lever responding in C57BL/6J mice, both of which occurred at doses which suppressed responding. These data provide additional evidence of the asymmetrical substitution of ethanol and NMDA antagonists (Grant, 1994).

In the aggregate, the results of tests with abused solvents and methoxyflurane suggest that the discriminative stimulus effect of these compounds are not dizocilpine-like and therefore may not be NMDA-receptor mediated. With the exception of 16,000 ppm TCE in C57BL/6J mice, which produced 33% dizocilpine-lever responding, none of the concentrations of TCE, toluene, xylene or methoxyflurane produced more than 19% dizocilpine-lever selection in either strain. This was despite testing response rate-suppressing concentrations of all four vapors. Indeed there was, on average, similar or even slightly greater substitution of pentobarbital for dizocilpine in DBA/2J and midazolam for dizocilpine in both C57BL/6J and DBA/2J mice than any of the volatile compounds. In a previous study in Swiss–Webster mice trained to discriminate 2 mg/kg phencyclidine from saline, 16,000 ppm TCE also produced only 30% phencyclidine-lever selection. However, in that same study, toluene produced 67% dizocilpine-lever selection and methoxyflurane 35% phencyclidine-lever selection, suggesting some degree of discriminative stimulus similarity between phencyclidine and these compounds (Bowen et al., 1999).

The present findings showing that positive $GABA_A$ modulators can produce as great, or perhaps slightly greater, dizocilpine-like discriminative stimulus effects than the tested vapors suggests that studies, such as Bowen et al. (1999) could have observed phencyclidine-like effects of solvents due to inherent similarity in the discriminative stimulus effects of phencyclidine and $GABA_A$-positive modulators. There are also a number of other possible reasons why the findings in the present study did not correspond with those previously reported. Firstly, since Swiss–Webster mice were used in the prior study and C57BL/6J and DBA/2J mice were used in the present

experiment, it is possible that the strain of mouse could have played a role in the differences noted. Although this hypothesis cannot be ruled out without testing toluene and methoxyflurane in Swiss–Webster mice trained to discriminate dizocilpine, this possibility seems somewhat unlikely given that there were no pronounced strain differences in the pattern of substitution of the volatile compounds in C57BL/6J and DBA/2J mice.

A second and more likely reason for the differences in substitution results revolves around the use of dizocilpine as a training stimulus in the present study compared to phencyclidine in the previous experiment. Although both dizocilpine and phencyclidine are uncompetitive NMDA antagonists, differences exist in their binding profiles as well as in downstream neurochemical effects. For instance, phencyclidine has been shown to bind to the dopamine uptake complex as well as the NMDA receptor, whereas dizocilpine does not have a similar dopamine uptake site binding profile (Maurice et al., 1991). Phencyclidine and dizocilpine also differ in their antagonism of NMDA-evoked dopamine, acetylcholine and spermidine release (Nankai et al., 1998; Snell et al., 1988). Lastly, phencyclidine but not dizocilpine has recently been shown to increase vesicular dopamine uptake (Crosby et al., 2002). These finding would suggest that dizocilpine might be a more selective NMDA antagonists than phencyclidine and therefore have a more narrowly defined discriminative stimulus.

Despite the fact that there were no pronounced strain differences in the substitution profiles of the volatile compounds and other drugs for dizocilpine, there were interesting differences in the operant response rate altering effects of these compounds between strains. Midazolam, but not phencyclidine, pentobarbital or ethanol, showed greater rate increasing and significantly less potent rate decreasing effects in C57BL/6J than DBA/2J mice. This finding is in accordance with the pattern of operant rate alterations resulting from midazolam, pentobarbital and ethanol in a prior ethanol drug discrimination study in C57BL/6J and DBA/2J mice (Shelton and Grant, 2002). Among the volatile compounds tested, toluene at 6000 ppm suppressed operant response rates to 41% of saline control levels in C57BL/6J mice, but almost completely suppressed responding in DBA/2J mice. In contrast, xylene at concentrations up to 6000 ppm had virtually no effect upon operant response rates in DBA/2J mice, but suppressed responding by more than 90% in C57BL/6J mice. Although relatively little is known about the differences in behavioral effects of volatile solvents, the present results suggest that operant responding in inbred mouse strains might be a valuable tool for differentiating between these compounds.

In summary, these results support the hypothesis that the discriminative stimulus effects of volatile solvents, at least TCE, toluene and xylene as well as the volatile anesthetic, methoxyflurane, may not be mediated by NMDA receptors. However, based on the patterns of operant response rate elevation and suppression in C57BL/6J and DBA/2J mice, it is clear that each of these volatile compounds can be differentiated based upon behavioral effects, suggesting that there may also be difference in their neurochemical sites of action. Additional studies will need to be conducted to further elucidate these differences as well as to more completely define the discriminative stimulus effects of these compounds.

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