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Toluene inhalation produces a conditioned place preference in rats

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Abstract

Toluene is a widely abused solvent with demonstrated addictive potential in humans. Here we explore if conditioned place preference can be used to study the abuse-related effects of inhaled toluene in rats. Animals were confined to a distinctive compartment of a three-compartment chamber while exposed to toluene vapor and later tested for preference for that compartment compared to appropriate control subjects. In this study, a flame ionization detector was used for on-line monitoring of toluene vapor concentrations inside the conditioning apparatus coupled with computerized recording of the time spent by the animals on the test day in each of the chambers. Sprague–Dawley rats were exposed to 810, 1895 or 4950 ppm of toluene vapors in either the back or white compartment during 30-min pairing sessions given every other day alternating with air exposure for the total of six pairings for each treatment. Rats that received air in both sides (control group) did not show any preference for either side with approximately equal time spent in each compartment on the test day (241 ± 33 and 234 ± 34 s, for white and black box, respectively). However, the 1895- and 4950-ppm test groups, but not the 810-ppm group, demonstrated a significant preference for the side paired with toluene exposure. When a subsequent test session was performed during toluene exposures, no conditioned place preference was observed. Thus, toluene produced a clear conditioned place preference that appears to be most evident when animals are not intoxicated. This procedure should be useful for further studies of the abuse-related effects of abused inhalants.

Keywords: Solvent; Conditioned-place preference; Addiction; (Rodent)

1. Introduction

Solvent abuse is a significant worldwide problem associated with serious health and social cost (Brouette and Anton, 2001; Howard and Jenson, 1999). The neurological and psychiatric consequences of inhalant abuse include paranoid psychosis (Byrne et al., 1991), parkinsonism (Uitti et al., 1994), cognitive impairment (Chadwick et al., 1989; Chadwick and Anderson, 1989) and cerebellar dysfunctions (Kamran and Bakshi, 1998; King, 1982). In addition, a history of inhalant abuse is associated with a substantially increased likelihood of adolescents to develop other substance abuse disorders (Dinwiddie et al., 1991a,b; Johnson et al., 1995; Schutz et al., 1994). Though inhalants produce many psychological and behavioral effects similar to those of other abused drugs and meet the DSM-IV diagnostic criteria for drug dependence (Howard et al., 2001; Kono et al., 2001; Miyata et al., 2001), the development of animal models for their abuse-related effects lags far behind progress with other classes of drugs of abuse (Balster, 1998).

Abused solvents are almost always inhaled by substance abusers, and their very rapid distribution into the brain (Gerasimov et al., 2002a) may result in immediate reinforcing effects which contributes to their abuse liability and risk of dependency. Therefore, animal models, which utilize the inhalation route, are important for studying the abuse-related reinforcing and other central nervous system effects of these agents. Undoubtedly, the technical difficulties of producing controlled exposures to vapors have been limiting the development of these animal models.

Place preference conditioning is a valuable, firmly established, and widely used tool in behavioral pharmacology and addiction research (for review, see Bardo and Bevins, 2000; Tzschentke, 1998). We have chosen to utilize the

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conditioned place preference paradigm to measure reward-related behaviors in rats exposed to toluene, the essential psychotropic component found in many abused solvents (Balster, 2003). To pursue this goal, we have recently developed a unique, completely automated, dynamic vapor exposure conditioned place preference apparatus to investigate behavioral effects of inhalants.

To our knowledge, there have been only two previous reports of using the conditioned place preference paradigm to study abused solvents, both also utilizing toluene. Yavich et al. (1994) exposed rats to mixtures of organic solvents containing 25% of toluene using a static vapor exposure chamber prior to placing them in the conditioning compartments. A more recent report describes a significant conditioned place preference induced in mice exposed to toluene vapors during conditioning in a dynamic two chamber apparatus (Funada et al., 2002). Because recovery from toluene exposures is so rapid, there are obvious advantages to conducting training session during the vapor exposures, therefore, this is the approach utilized in the present study.

We examined the rewarding properties of toluene (810, 1895, and 4950 ppm) in rats using a conditioned place preference paradigm with a three-compartment chamber. The choice of this dose range was based on the report by Bowen et al. (1999) showing that 600–1000 ppm is the minimal effective concentration for producing toluene's discriminative stimulus effects in mice. Accordingly, 10,000–12,000 ppm produces the symptoms of ataxia, slurred speech and hallucinations in toluene abusers (Garriott et al., 1981; King, 1982). However, in our hands, exposure to 10,000 ppm for 15 min produced severe ataxia and even the loss of conscience in animals.

Treatment animals were exposed to toluene in a distinctive compartment during conditioning, while control animals received the same experiences in the apparatus, but without exposures to toluene (air only). During test sessions, animals were initially placed in a middle compartment and allowed access to all three chambers. A preference for the toluene-paired compartment was used to measure the conditioned place preference. Because the expression of a conditioned place preference can sometimes be greater when animals are tested when under the influence of the training drug (Bespalov et al., 1999), we compared test sessions conducted with and without toluene exposure. In addition, we combined the conditioned place preference paradigm with measurements of locomotor activity by recording the number of chamber crossings during the test sessions.

2. Materials and methods

2.1. Subjects

All animals use procedures were in strict accordance with the National Institute of Health guide for the care and use of all laboratory animals and were approved by the local animal care and use committee. Studies utilized experimentally naïve, male, Sprague–Dawley rats ($n = 8$ per group, total of 48 animals; Taconic Farms, Germantown, NY that weighed 150–250 g) and were given food and water ad libitum while housed in a vivarium. Temperature and humidity were kept relatively constant, and all animals were housed in pairs and kept on a 12/12-light/dark cycle. Training and testing were conducted during the light cycle.

2.2. Conditioned place preference apparatus

The conditioning apparatus (ENV-013, MedAssociates, St. Albans, VT) consisted of three distinct compartments separated by two guillotine doors. The walls of the middle chamber were gray with a smooth floor, while one conditioning compartment ($21 \times 21 \times 27.5$ cm, internal volume 12 l) had black walls with a smooth floor and the other one had white walls with a steel mesh floor. The lids of all three compartments were made of transparent plexiglass. The time spent in every compartment during test sessions was automatically recorded by infrared photocells positioned along the walls at the level of animal's head (six on both sides of the two conditioning compartments and three on both sides of the middle compartment) interfaced with MED-PC for Windows Version IV and Delphi TM 4 (SOF-735) (MedAssociates). The beams were arranged such that when an animal enters a chamber (defined as forepaws and the entire head in the chamber), the beam is broken and the timer begins recording. Once the animal leaves the chamber, the timer stops. In addition, the circuitry has an internal timer that shuts off all the beams after 15 min, which is the duration of the test session. The response of the computer to the break of a beam by an animal's body occurs within a tenth of a second. Accordingly, as an animal moves along from one beam to another the recording of the new position starts as the recording of the old position is ended. The cumulative data are presented as the number of seconds (tenths of seconds were eventually rounded for the clarity) spent in each chamber.

The apparatus was modified to allow for dynamic vapor exposure. To render the exposure chambers airtight, the lids were equipped with rubber gaskets and clasp locks. An opening on the top of the side of both black and white compartments was used to introduce toluene vapors under positive pressure, and the atmosphere was exhausted through an opening at the bottom of both chambers, insuring that atmospheric pressure within the chamber was not altered by the introduction of the toluene vapor. The flow of vapors was initiated at least an hour prior to the beginning of the exposure to allow for chamber concentrations to equilibrate, as confirmed by air sampling (see below). Animals were quickly introduced into the chambers by opening the lid, which was immediately closed and the chamber resealed while toluene vapors continued to be introduced for the remaining of conditioning session.

2.3. Measurement of toluene concentrations in conditioned place preference apparatus

Toluene (99%) was purchased from Sigma-Aldrich (Milwaukee, WI). Mixtures of toluene vapors and air were metered using two mass flow controllers (Dyna-Blender by Matheson Tri-Gas, Mongomerville, PA) with a total flow of gas mixture set at 2 l/min. Pure laboratory air was metered through one flow controller while the second one metered air that was bubbled through a 500-ml round bottom flask filled with toluene set either in a common flask holder (room temperature) or in an insulated ice bath (used only for creating 800-ppm vapor levels). All connections were made using 0.25-in. outside diameter stainless steel tubing. Blending the two gas streams created variable concentrations of toluene vapors that were continuously monitored by partitioning a small portion of the gas stream through a 1/32-in. outside diameter stainless steel tube interfaced with a flame ionization detector (SRI Instruments, Torrance, CA). The signal output from this detector was analyzed using PeakSimple software (SRI Instruments).

The on-line flame ionization detector response to various levels of toluene vapors was calibrated using a capillary gas chromatograph (Hewlett Packard 5690A) equipped with a 60×0.25-mm i.d. SE-30 capillary column maintained initially at 50 °C, with the temperature programmed to reach 150 °C at 3 °C/min rate at the time of sample injection. Helium was used as a carrier gas.

For calibration purposes, toluene vapor samples were collected from the tubing outlet connected to the chambers by bubbling the gas stream through a vial with 10 ml of hexane for 2 min. To insure that trapping of toluene was quantitative, parallel measurements were obtained using 100-ml vials of hexane with collection periods increased to 10 min. No differences were noted between the two sampling times, suggesting that all toluene was trapped in the 2-min sample. This also then served as a systematic replication of the calibration curves.

Toluene peaks were analyzed and integrated using a Vision 4 Chromatography Acquisition station. The integrated peaks (in peak-area-units) were subjected to a linear regression analysis and the resulting equation was used to convert peak-area-units to nM and subsequently parts per million (ppm) of toluene.

In order to independently verify toluene levels created in the exposure chambers and insure that the levels were uniform, nine small holes (three for each level: top of the chamber, level of animal's head, and 2 cm above the floor) were drilled in the walls of both boxes. Air samples were drawn with a gas-tight syringe and were immediately dispensed into vials containing hexanes to trap the toluene vapors. The amount of toluene was then determined from the calibration curve.

Mixing the gas streams of toluene and pure air in the proportion of 1.0 l/min of pure air and 1.0 l/min of toluene kept at room temperature yielded an average toluene concentration inside the chamber of 4950 ppm. A mixture of 1.5 l/min of pure air and 0.5 l/min of toluene produced an average toluene concentration of 1895 ppm. We obtained an average toluene vapor concentration of 810 ppm with a mixture of 1.95 l/min pure air and 0.05 l/min of toluene kept at 0 °C to retard evaporation.

It should be noted here that the actual concentrations of toluene vapors obtained inside the chamber are the function of the step-wise changes in the ratio of two gas flows dictated by the design of the flow meters used in our experiments.

2.4. Procedures

Pre-conditioning phase: during the first two pre-conditioning days, the animals were transported from the Brookhaven Laboratory Animal Facility to the Chemistry Department building in order to get acclimated to the transportation procedure and to the test room. The animals were transported daily at 12 pm in their home cages, two animals per cage. On the third pre-conditioning day, the animals were allowed to freely explore all three compartments of the conditioned place preference chamber for 15 min and the time spent in each compartment was electronically recorded to assess the unconditioned preference for either one of the distinctive side compartments. On average, the animals spent approximately an equal amount of time (mean $\pm$ S.E.M. seconds) in both white and black chambers (black: 286 ± 22; white: 291 ± 26) in this pre-conditioning session. Thus, the apparatus was truly unbiased in terms of chamber preferences in untreated rats.

Conditioning phase: during the following conditioning phase (12 days, six pairings for each treatment, consecutive days, including weekends), rats were assigned to receive toluene (810, 1895 or 4950 ppm) or air in one of the two compartments in a counterbalanced fashion, with half of the animals receiving toluene in the white compartment and air in the black one and the other half receiving toluene and air in an opposite fashion. During each training session, animals were confined for 30 min to one side of the apparatus with the doors closed. Cage mates were exposed to the training drug in pairs, so that on any given conditioning day, both sides of the apparatus were filled with either air or toluene. The control group was exposed to air in both compartments. Fecal pellet output rate in each compartment was used as an indicator of stress and recorded at the end of each session. The last day before the test day was always assigned to be an air exposure conditioning session.

Testing phase: on the first test day (the next day after the last training session), animals were placed in the middle compartment of the conditioned place preference chamber for 5 min with the guillotine doors closed for an initial acclimation. Subsequently, the doors were raised and the animals were allowed free access to all three compartments for 15 min. The time spent in each compartment (white/gray/black) was electronically recorded. A locomotor activ-

ity score was obtained by recording the total number of chamber crossing. Due to technical problems, these scores were obtained only in the animals in the 1895- and 810-ppm toluene groups.

On the second test day, the animals were brought back and again allowed free access to all three chambers. However, this time, the apparatus was filled with the training concentration of toluene. The control group of toluene-naïve animals was pre-conditioned and tested under an identical protocol, except that toluene was omitted on conditioning sessions.

2.5. Statistical analysis

Conditioned place preference test outcomes for each concentration were determined by the time spent in the chamber paired with toluene compared to the chamber paired with air. A positive conditioned place preference for a particular dose of toluene was indicated by a significantly ($p < 0.05$, paired two-tail t-test) greater mean time spent on the toluene-paired side than on the air-paired side. For the locomotion data, the number of chamber transitions on the first test day was compared for the same group of animals with the number of crossings on the second test day when toluene was administered.

3. Results

3.1. Conditioned place preference

Rats that received air in both sides (control group) did not show any preference ($p = 0.8$) for either black or white side with approximately equal time spent in the back and white compartments on the test day (mean ± S.E.M. of 241 ± 33 and 234 ± 34 s, respectively). These results in the air control group are very similar to the results for all the animals when their preference was measured in the pre-conditioning phase (black: 286 ± 22; white: 291 ± 26, $p = 0.7$).

Animals conditioned with 810 ppm of toluene did not show a significant preference ($p = 0.7$) for any compartment (274 ± 24 vs. 261 ± 26 s spent on toluene and air-paired side, respectively) (Fig. 1A, left panel). Exposure to this concentration of toluene on the second test day did not alter this outcome (291 ± 31 vs. 302 ± 39 s) (Fig. 1A, right panel).

On the other hand, animals conditioned with 1895 and 4950 ppm of toluene spent significantly ($p < 0.05$) more time on the toluene-paired side than on the air-paired side (299 ± 26 vs. 201 ± 22 s and 306 ± 29 vs. 208 ± 19 s, for these two concentrations, respectively) (Fig. 1B and C, left panels). Exposure to the training concentration of 1895 ppm on the second test day led to the loss of preference for the toluene-paired side in this group of animals. In fact, we observed a trend for an increase in the time spent on the air-paired side that did not reach significance ($p = 0.2$) (215 ± 66 vs. 406 ± 94 s for toluene and air side, respectively) (Fig.

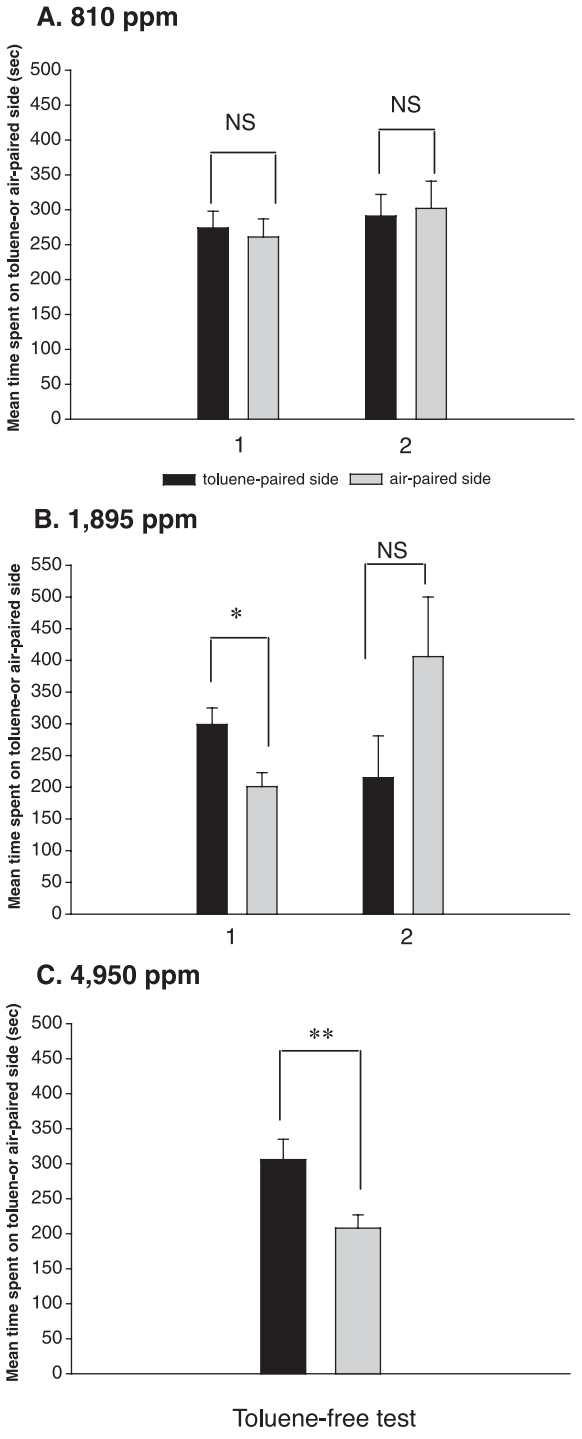

Fig. 1. Results of tests for a conditioned place preference with three concentrations of toluene. Shown is the average time spent in the toluene or air-paired side on test day 1 (toluene-free) and on test day 2 (during exposure to the training concentration of toluene). (A) Animals conditioned with 810 ppm of toluene; (B) Animals conditioned with 1895 ppm of toluene; (C) Animals conditioned with 4950 ppm of toluene. Data are means ± S.E.M. of eight rats per group. NS, not significant using a paired t-test, *$p < 0.05$, **$p < 0.01$.

1B, right panel). A second test session with toluene exposure was not conducted with the 4950-ppm concentration.

3.2. Locomotor activity

There was no difference between chamber crossings in test sessions conducted with and without toluene in either the toluene-conditioned animals ($p = 0.14$) or toluene-naïve animals receiving toluene for the first time ($p = 0.06$) (Fig. 2A). In the group of animals trained with 1895 ppm of toluene, locomotor activity was significantly ($p < 0.01$) lower when the animals were tested when exposed to toluene, as compared to their score obtained during the subsequent toluene-free test session (Fig. 2B, left panel). However, no significant difference ($p = 0.47$) was observed between toluene-free and toluene-exposed test sessions in the toluene-naïve animals (Fig. 2B, right panel).

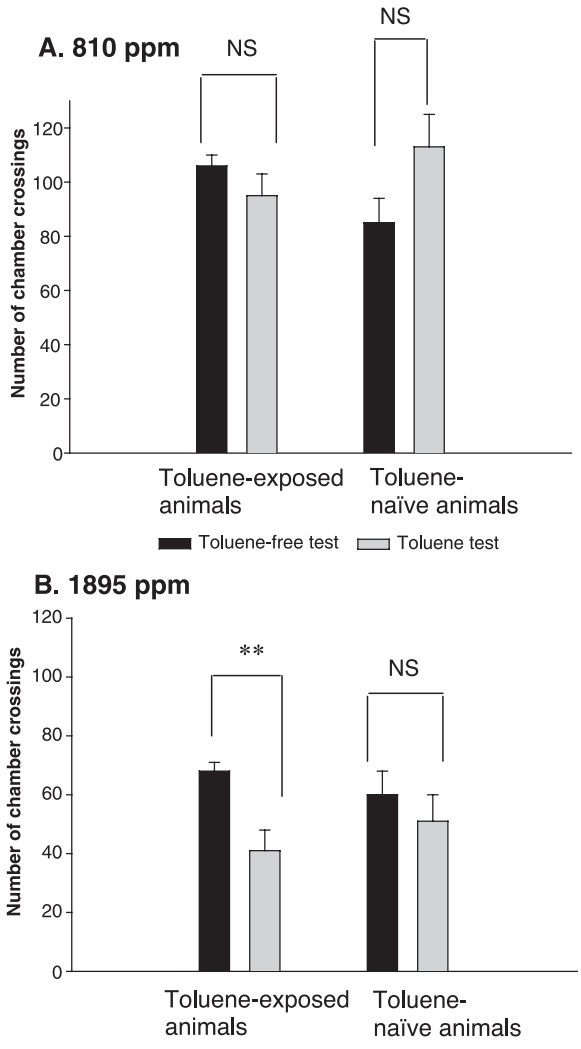

Fig. 2. Effects of two concentrations of toluene on locomotor activity during test sessions. Shown are the number of chamber transitions on test day 1 (toluene-free) (left panels) and on test day 2 (during exposure to the training concentration of toluene) (right panels) for toluene-trained and toluene-naïve rats. (A) Animals conditioned with 810 ppm of toluene; (B) Animals conditioned with 1895 ppm of toluene. Data are means ± S.E.M. of eight rats per group. NS, not significant using a paired *t*-test, $^{**}p < 0.01$.

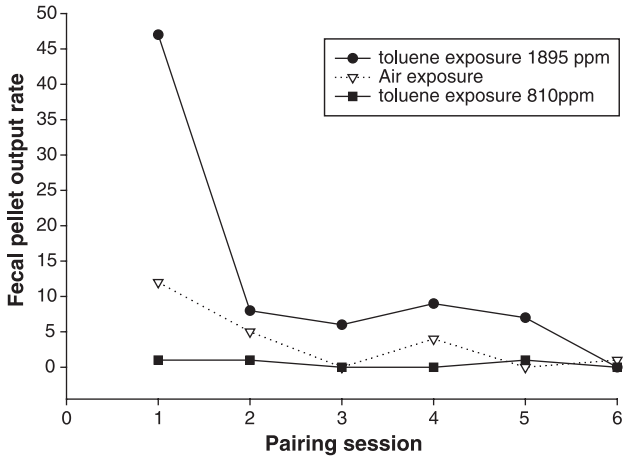

Fig. 3. Stress response to toluene (810 or 1895 ppm) or air during the training sessions. Fecal pellets were collected after each conditioning session and counted. Data are the total number of pellets for eight animals on a given day.

There were significant differences between test sessions conducted with 810-ppm toluene exposure and test sessions with 1895-ppm exposures. The number of chamber crossings was lower in the 1895-ppm exposures than with the 800-ppm exposures in both trained and naïve animals ($p = 0.002$ for both groups) (compare upper and lower panels of Fig. 2).

A stressful response (represented by fecal pellet output rate) in the animals exposed to 1895 ppm was observed on the first day of toluene conditioning which showed adaptation by the second pairing session (Fig. 3). Animals conditioned with 810 ppm of toluene did not display any changes in pellet output throughout the pairing sessions compared with the air-trained control animals.

4. Discussion

Toluene is considered to be a major chemical constituent contributing to the morbidity associated with solvent abuse. Unrestricted availability, low cost, ease of use, and rapid intense effects all contribute to the popularity of this substance among children and adolescents seeking experience with mood-altering drugs (Mackesy-Amiti and Fendrich, 1999; McGarvey et al., 1999; Morita et al., 1994). An important goal of this study was to further develop an animal model for some of the abuse-related effects of this solvent.

The major result of the present study was that a concentration-dependent conditioned place preference could be developed in a rat model which has been utilized extensively for studying other classes of drugs of abuse. This represents the first reported study in rats using pure toluene administered during conditioning sessions by inhalation and

extends the results from the previous two reports of toluene-induced conditioned place preference in mice (Funada et al., 2002) and in rats (Yavich et al., 1994).

Conditioning sessions performed with 1895- and 4950-ppm toluene produced a significant conditioned place preference. The degree of preference observed here is similar to the mean conditioning score (the difference between the time spent on the paired vs. unpaired side) of 180, 140, and 150 s previously reported for the group of mice exposed to 700, 2500, and 3200 ppm of toluene, respectively (Funada et al., 2002). This range of effective concentrations (2000–5000 ppm) corresponds to other studies of the behavioral effects of toluene in laboratory animals. These include changes in locomotor activity in mice (Bowen and Balster, 1998), cross-sensitization to the locomotor-stimulating effects of cocaine in rats (Beyer et al., 2001), direct effects on schedule-controlled responding in mice (Moser and Balster, 1985; Bowen and Balster, 1998), discriminative stimulus effects in mice and rats (Rees et al., 1987; Yavich et al., 1994), self-administration in monkeys (Weiss et al., 1979) and mice (Blokhina et al., 2001) and in decreased intracranial self-stimulation current intensity threshold in rats (Bespalov et al., 2003). It should be noted here that in the latter study, toluene vapors (3600 ppm) facilitated electrical self-stimulation behavior without affecting the responding at the "reset" side, thus indicating that toluene at this concentration might sensitize the dopaminergic mesocorticolimbic pathway to electrical stimulation. Accordingly, our own findings indicate that 3000 ppm of inhaled toluene produces regionally specific changes in extracellular brain dopamine levels in rats (Gerasimov et al., 2002b).

In the present study, the lower dose of 810-ppm toluene failed to produce a conditioned place preference. Thus, it is not the odor of toluene alone that produces the conditioned place preference, since 810 ppm is strongly odiferous. Indeed, it is likely that this concentration is below the threshold for producing effects in the brain necessary for developing a basis for the conditioned place preference. However, this finding should be considered in the context of species differences. That is, numerous behavioral studies in mice show that 600–1000 ppm of toluene is still effective in producing discriminative stimulus effects (Bowen et al., 1999), effects on locomotor activity and schedule-controlled behavior (Bowen and Balster, 1998), and in anxiety paradigms (Lopez-Rubaclava et al., 2000). Finally, Funada et al. (2002) observed a significant conditioned place preference in mice exposed to 700 ppm. Species differences between mice and rats have been reported before, for example, another abused "solvent" ethanol produces conditioned place aversion in rats and a conditioned place preference in mice (Cunningham et al., 1993). One might argue that rats are less sensitive than mice to the central nervous system effects of toluene possibly due to noted differences in toluene effects on dopaminergic system between the two species (for review, see Von Euler, 1994).

We found that the toluene conditioned place preference was only evident on test sessions when toluene was not administered, and that it disappeared when animals were retested while simultaneously exposed to toluene. To date, relatively few studies have evaluated how drug administration during testing sessions affects the expression of preference (or aversion) following place conditioning and most studies utilize only a drug-free testing condition. For example, relative preference or aversion (relative with respect to novelty) produced by two different doses of amphetamine was only expressed in animals tested under the influence of the conditioning drug (Laviola and Adriani, 1998). Interestingly, in the report by Bespalov et al. (1999), pretreatment of mice with morphine led to a dose-dependent increase in the time spent in the morphine-paired compartment, with the maximum effect observed when the same doses were used for the training and testing sessions. This finding contrasts with our study in which not only did the testing in the presence of toluene exposure (1895 ppm) not enhance the expression of the conditioned place preference, but instead actually decreased the preference for the toluene-paired compartment. The basis for this decreased expression is not known at the present time. One possible explanation is based on the report that the *n*-methyl-D-aspartate (NMDA) antagonist dizocilpine interferes with the expression, but not the acquisition, of bromocriptine-induced sensitization (Carlezon et al., 1995) and that NMDA antagonists in general interfere with the expression of learned drug-environment associations (Bespalov et al., 2000). Experimental evidence that toluene acts as a noncompetitive NMDA antagonist in vitro (Cruz et al., 1998) and in vivo (Cruz et al., 2003) leads to the hypothesis that testing during toluene exposures interferes with the retrieval of the association between toluene and the distinctive compartment.

It should also be noted that the tests with toluene exposure occurred after tests in the toluene-free condition without any intervening training. Thus, there is an order effect, which confounds interpretation of these test session differences. For example, the loss of the conditioned place preference in the second test session could simply reflect extinction of the toluene-environment association. However, more research is needed to clarify the potential mechanism of state dependency in toluene-induced conditioned place preference and we are currently performing additional experiments to address this important issue.

Our data indicate that the number of transitions from one chamber to another during the test session was significantly lower when the animals were exposed to the training concentration of 1895 ppm as compared to their locomotion during the drug-free (air only) test. At first glance, this is not surprising, since toluene has been reported to increase spontaneous locomotor activity at lower doses and to decrease activity at higher exposure levels (3000–6000 ppm) in mice (Wood and Colotla, 1990). In addition, Kjellstrand et al. (1985) have reported that exposure of mice to a strongly scented cologne had no effect on motor activity, suggesting

that the effects of solvents on locomotor activity are not likely due to their odoriferous properties. However, there is one important caveat that should be considered here. The decrease in locomotor activity induced by exposure to 1895-ppm toluene relative to air test conditions was observed only in the paired (treated) group of animals, but not in the control, toluene-naive group. It appears then that the toluene-paired animals were more sensitive to locomotor suppressing effects of toluene than toluene-naïve animals. This is consistent with the concept of sensitization, previously reported for other drugs of abuse (for review, see Kalivas, 1992; Kalivas and Stewart, 1991; Kalivas et al., 1992) and for toluene itself (Beyer et al., 2001).

Finally, it may initially be difficult to reconcile the stressful effects of acute toluene exposure as evidenced by an increased rate of fecal pellet output (see Fig. 3) with other evidence that it has anti-anxiety effects in mice in the dose range of 1000–4000 ppm (Bowen et al., 1996; Lopez-Rubaclava et al., 2000). It could be hypothesized that, with repeated training sessions, animals habituate to the aversive effects, as evidenced here by the return to control values of fecal boli already by the second training session (Fig. 3).

In conclusion, we have successfully demonstrated that the conditioned place preference paradigm can be used to study abuse-related behaviors of toluene. Because toluene shares many other abuse-related properties with abused solvents (Balster, 1998), it should be possible to apply this paradigm to the study of other inhalants as well. Toluene's conditioned place preference effects were concentration related and occurred in a concentration range consistent with previous behavioral and neurochemical reports. The conditioned place preference model should be very useful for evaluating the abuse potential of various solvents and studying the behavioral and neural bases for their abuse-related effects.

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