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Effect of Xylene Inhalation on Fixed-Ratio Responding in Rats

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GHOSH, T. K., R. L. COPELAND, JR., R. N. PARUI, S. MOOKHERJEE AND S. N. PRADHAN. *Effect of xylene inhalation on fixed-ratio responding in rats.* PHARMACOL BIOCHEM BEHAV **27**(4) 653–657, 1987.—The effect of xylene inhalation was studied on operant behavior under a fixed-ratio (FR24) schedule in rats. Experiments were performed while rats were being exposed to xylene vapor in an inhalational (flow-through) behavioral chamber. Rats were exposed successively to three graded concentrations (113, 216 and 430 ppm) of xylene vapor each for 2 hr in range-finding studies during 6¼-hr sessions. The reinforcement rate which is correlated with FR responding was shown to be decreased at hr 1, hr 3 and hr 5. However at hr 2, hr 4 and hr 6 the reinforcement rate in rats increased approaching the control levels, thereby indicating development of tolerance. When rats were exposed to one of the three graded concentrations of xylene for 2 hr on separate days, they also showed a decrease in the reinforcement rate at hr 1; development of acute tolerance was also noted in this schedule. Exposure to the lowest (98.5 ppm) level of xylene used during 5-hr sessions caused no significant decrease in the reinforcement rate. This study thus attempts to identify a minimum effective concentration of xylene and indicates the development of acute tolerance to behavioral effect of xylene.

Xylene Fixed-ratio responding Tolerance Inhalational chamber

EXPOSURE to organic solvents may occur in the work place and is also a form of substance abuse. Substances which have been abused via the inhalation route include plastic cement, paint thinner, gasoline, airplane glue, nail polish remover, etc. [2, 23, 27]. These substances contain toluene, acetone, xylene, benzene, ethyl ether, alcohol and others.

Behavioral techniques are sensitive and in many instances may detect toxic effects that may not be evident through gross pathological observations. Many studies on behavioral effects of inhaled solvents have utilized a static (closed) or dynamic (flow-through) exposure system where animals are placed in a chamber with a known concentration of volatilized solvent (e.g., [1, 8, 10, 13, 24, 26]). Some of the researchers performed the behavioral testing after the animal was removed from the exposure chamber (e.g., [13, 24, 26]). The major disadvantage of this procedure is the rapid recovery of function towards the normal level once the animal has been removed from the chamber. On the other hand, some have investigated the behavioral effects of volatilized solvents during static or dynamic exposure (e.g., [1, 8, 10, 15]). These procedures may be more suitable for inhalation toxicology experiments.

Xylene is widely used in industry as a degreasing agent or as a chemical intermediate and in histological laboratories. Exposure to high concentrations of xylene produced anesthesia and even death in animals (5100 ppm) and man (10,000 ppm) [3, 12, 25]. Human subjects exposed to xylene vapor at low concentrations showed impairment of psychophysiological functions such as numerical ability, reaction time, body balance and critical fusion frequency [7, 17, 19–22]. Behavior of laboratory animals has been considered in the assessment of the effect of xylene on the central nervous system (CNS). The learning process of the rat in a maze task was retarded during daily subcutaneous injection of 0.5 ml xylene [4]. A transient decrease of the preening frequency in the rat was reported during a long-term intermittent exposure to 300 ppm of xylene [18]. Motor performance of mice on an inverted screen test was disrupted by xylene at 3000 ppm [13].

Operant behavior, which is a sensitive measure for the detection of CNS effects at a minimal concentration of substances abused through inhalation [8], has been used in few studies of the behavioral pharmacology of xylene. Responding of rats under a fixed-interval (FI) schedule was both increased and decreased in a time-dependent manner after 2-hr exposure to 560–3000 ppm of xylene [9]. Operant performance of mice under differential reinforcement at low rate (DRL) schedule was impaired immediately after 30 min of exposure to 1400 ppm of xylene [13]. Behavioral performance was measured in both of these studies after the exposure was over. Therefore, magnitude and temporal patterns of xylene effects on schedule-controlled behavior cannot be examined. A dynamic inhalation behavioral chamber has

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been developed in this laboratory [16], which can be utilized to measure the schedule-controlled behavior during and following exposure to inhaled organic solvent vapor. Different schedules of reinforcement (fixed-ratio, fixed-interval, DRL and different combination of these) have been used by several investigators to assess the effects of inhaled solvents [8, 10, 11, 13, 14]. In the present study we examined the effect of xylene inhalation at low concentrations on operant performance under fixed-ratio schedule in rats and an attempt was made to determine the minimum effective concentration (MEC) for any such effect.

METHOD

Animals

Test animals consisted of male F344 rats (Charles-River Breeding Lab) having initial body weight of 210–225 g. These rats were maintained at 80% of their original starting weights by controlled post-session feeding. Animals were housed individually in stainless steel wire mesh cages and were kept in artificial light-dark cycle of 12 hr, and the mean±S.E. of the temperature and relative humidity of the animal room were 21.1±0.87°C and 63.1±7.5% respectively.

Chemical

Rats were exposed to the vapor of xylene (laboratory grade, Fisher Scientific Co.) which is a mixture of meta-, para-, and ortho-xylene and contains some ethylbenzene as a contaminant.

Apparatus

The design of the inhalational behavioral chamber has been described in detail elsewhere [16]. Briefly described, the chamber consisted of an inverted cylindrical glass chromatography jar which covered a circular grid floor with a vertical metal plate accommodating a lever and a liquid dipper. The lever was connected to a microcomputer by wires passing through the floor of the chamber and its pressing would allow the animal to be reinforced with a dip (0.2 ml) of a liquid.

The chamber was infused with a flow of air derived from the house air supply. This air was first filtered to remove dust, oil particles, and other contaminants and passed through a pressure regulator and a gas flow-meter. The xylene vapor was generated by injecting the volatile liquid with a 1-ml gas-tight Teflon-tipped syringe driven by an infusion pump into an evaporating flask where the vapor mixed with the filtered house air flow. The vapor mixture passed through a condensor set at a constant temperature (22°C), to lower the temperature of this mixture before being infused into the chamber. The vapor mixture was introduced into the chamber through a cross-shaped copper tubing system suspended from the ceiling of the chamber, so that it was infused downward into the chamber to produce a homogenous distribution.

Gas samples were collected at 15-min intervals from inside the chamber through a metal tube located underneath the floor of the chamber into a gas sampling bulb. One ml of the vapor sample was injected into a Shimadzu dual-column gas chromatograph (Model GC Mini 2) equipped with flame ionization detectors. Silanized glass columns (3.5 meter, 3 mm i.d.) were packed with GP 5% SP-1200/5% Bentone 34 on 100/120 Supelcoport. Gas flow for the GC was maintained at N_2 500 ml/min, air 450 ml/min, and H_2 40 ml/min. Column temperature was maintained at 80°C and the injector and detector temperatures were 110°C. A digital integrator was employed to measure the concentration of xylene and the average concentration based on the samples was expressed as mean±S.E.

TABLE 1

EFFECT OF XYLENE INHALATION ON THE REINFORCEMENT RATE IN RATS RESPONDING UNDER A FIXED-RATIO (FR-24) SCHEDULE

Concentration (ppm)	Hours	Reinforcement/15 Min at Each Hour of Experiment* Previous Day Control	Experimental Day	% Change
Control (0)	0	30.8 ± 3.0	30.0 ± 3.1	−2.5
Low (113.1 ± 5.0)	1	31.3 ± 2.9	24.5 ± 2.6†	−21.7
	2	35.8 ± 4.6	30.8 ± 2.8	−14.0
Medium (215.7 ± 9.2)	3	36.0 ± 6.0	24.8 ± 3.5†	−31.2
	4	33.6 ± 5.3	35.6 ± 4.7	+6.0
High (430.3 ± 8.5)	5	36.6 ± 5.0	16.3 ± 2.4†	−55.5
	6	35.5 ± 3.5	34.0 ± 5.4	−4.2

*Mean ± S.E. for 4 rats.
†Significant ($p<0.05$).

Behavioral Schedule

Rats were trained to press the lever 24 times (FR24) to receive 0.2 ml of 5% sucrose solution. An arbitrary FR24 schedule was selected to shorten the behavioral training time in comparison to that for a higher FR schedule as well as to prevent the animals from reaching satiety quickly. When the performance of the animal reached a stable level during an 1-hr session (approximately after 6–8 weeks of training), they were divided in three groups and subsequently trained under different protocols. Behavioral sessions under these protocols started with a non-contingent delivery of sucrose solution which was given by manipulation of an external switch connected to the microcomputer. The animal did not get any reinforcement at the end of session.

In all experiments recordings were made for both responses and reinforcers which are roughly proportional. For calculation and evaluation purposes, reinforcement rate has been taken into consideration in these experiments.

Experiment I: Xylene Range-Finding Experiment

Four rats were trained for 6¼-hr sessions during which the lever pressing for the initial 15-min period was rewarded and recorded as control. Following this, lever pressing was rewarded during the last 15-min period (but not the initial 45-min period) of each hour for 6 hours.

After training for 10–12 daily sessions when lever pressing of rats was stabilized, behavioral sessions were carried out inside the inhalation behavioral chamber for further training and acclimatization of animals in the chamber environment where the final experiments on xylene exposure were to be performed. After 1–2 weeks of training when the stable per-

TABLE 2

CHANGES IN THE REINFORCEMENT RATE OF RATS RESPONDING UNDER A FIXED-RATIO (FR-24) SCHEDULE DURING 2-HOUR EXPOSURE TO XYLENE

Concentration (ppm)		Reinforcement/15-Min Session During Xylene Exposure*				
		Control	15–30 Min	45–60 Min	75–90 Min	105–120 Min
Low (113.8 ± 5.5)	A	48.0 ± 2.6	42.4 ± 2.8	38.0 ± 2.9	35.6 ± 4.9	27.6 ± 2.2
	B	48.2 ± 3.7 (+0.4)	41.2 ± 3.3 (−2.8)	30.2 ± 4.1† (−20.5)	36.0 ± 2.8 (+1.1)	28.0 ± 2.7 (+1.4)
Medium (212.0 ± 2.4)	A	59.4 ± 5.6	48.2 ± 4.9	46.0 ± 3.7	42.0 ± 5.1	38.8 ± 1.8
	B	55.8 ± 5.1 (−6.0)	43.8 ± 6.1 (−9.1)	33.6 ± 6.1† (−26.9)	37.2 ± 5.5 (−11.4)	38.0 ± 5.6 (−2.0)
High (445.8 ± 12.9)	A	48.2 ± 5.6	44.8 ± 4.9	37.8 ± 4.3	33.0 ± 1.8	31.0 ± 2.34
	B	49.8 ± 5.5 (+3.3)	44.6 ± 3.4 (−0.4)	29.0 ± 3.1† (−23.2)	26.8 ± 3.5 (−18.8)	25.6 ± 3.7 (−17.4)

*Mean ± S.E. for 5 animals. A: previous day control data; B: experimental day data; values within parentheses: % change from previous day control.
†$p<0.05$.

formance was achieved, the reinforcement rate for 3–4 subsequent days was recorded before rats were exposed to 3 graded concentrations of xylene (113.1±5.0, 215.7±9.2, 430.3±8.5 ppm) each of which was continued for 2 hr. Exposure to xylene was started following a 15-min control period.

Experiment II: Two-Hour Xylene Experiment

Five rats were trained for a 2¼-hr session during which behavioral performance was restricted to 2nd and 4th 15-min periods (i.e., 15–30 min and 45–60 min) of each hour. These rats were then transferred to the inhalational chamber where training was continued for 1–2 weeks. After the recording the stabilized reinforcement rate for 3–4 successive days, all rats were exposed to three graded concentrations of xylene (113.8±5.5, 212±2.4, 445.8±12.9 ppm) for 2 hr. Xylene concentrations were given in ascending order on three different days and to prevent tolerance development the same rat was not exposed to the next higher concentration of xylene for at least 7 days. Exposure to xylene was started following a 15-min control period. Thus reinforcement was available on five episodes during each session.

Experiment III: Five-Hour Exposure to the Lowest Level of Xylene

Four rats were trained for a session of 5¼ hr during which behavioral performance was limited to the last 15 min of each hour. After control studies in the inhalational behavioral chamber rats were exposed to 98.5±2.5 ppm of xylene for 5 hr. A 15-min control behavioral performance was recorded before xylene exposure as in the two previous experiments.

Statistical Analysis

Differences of reinforcement rates between pre-exposure control and each exposure period were determined for each animal on both the previous day control and the experimental day. These differences for corresponding periods of the previous day control and experimental day were analyzed by ANOVA using SAS general linear model procedure, modelling the within-period exposure-control comparison to determine the significant effect at an overall level of $p<0.05$.

RESULTS

Xylene Range Finding Experiment

Following exposure to xylene the reinforcement rate was found to decrease significantly ($p<0.05$) during the 1st, 3rd and 5th hours (Table 1). The decrease in the reinforcement rate was dependent on xylene concentration. The reinforcement rate decreased only by 22% at the low concentration and by 31% and 55% at medium and high concentrations respectively. However the FR performance at 2nd, 4th and 6th hours did not show any significant change. Reinforcement rate showed a 14% decrease at the 2nd hour while at the 4th hour 6% increase was noted. Only 4% decrease of reinforcement was noted at the 6th hour.

Two-Hour Xylene Experiment

During the 2nd 15-min (15–30 min) period of exposure to xylene there was no change of the reinforcement rate at all three concentration levels. However, during the 4th 15-min period (45–60 min) a significant ($p<0.05$) decrease was noted in the reinforcement rate at the three concentrations (Table 2). Schedule-controlled performance showed 20%, 27% and 23% decrease during 4th 15-min period (45–60 min) of exposure at low, medium and high concentrations of xylene respectively. The reinforcement rate at the 2nd hour of exposure showed no significant change with any concentration of xylene. But it was noted that during exposure to the high concentration (446 ppm) of xylene, the reinforcement rate during 2nd (75–90 min) and 4th (105–120 min) 15-min periods of 2nd hour showed 19% and 17% decrease respectively.

TABLE 3

CHANGES IN THE REINFORCEMENT RATE OF RATS RESPONDING UNDER A FIXED-RATIO (FR-24) SCHEDULE DURING 5-HR EXPOSURE TO A LOW CONCENTRATION OF XYLENE

Hour	Reinforcement/15 Min at Each Hour of Experiment* Previous Day Control	Experimental Day
0	34.2 ± 5.1	33.0 ± 4.9
1	33.0 ± 4.7	30.2 ± 5.8
2	35.0 ± 6.0	31.7 ± 5.6
3	36.0 ± 7.5	34.7 ± 6.1
4	37.2 ± 4.9	36.5 ± 4.0
5	35.5 ± 5.2	36.7 ± 4.9

*Mean ± S.E. for 4 rats. Animals were exposed to a mean concentration 98.5 ± 2.5 ppm.

Five-Hour Exposure to Xylene

The reinforcement rate was not significantly changed at any time during the five-hour exposure period at a very low concentration (98.5±2.5 ppm) when compared to previous day control (Table 3). No cumulative effect during prolonged exposure to this low concentration was observed in this experiment.

DISCUSSION

The inhalational behavioral chamber used in these experiments facilitated our investigation of behavioral effects of inhaled solvents (e.g., xylene) in rats. The dynamic exposure system used in the chamber offers certain advantages over the static system, as described by Balster *et al.* [1] and Glowa *et al.* [11]. Carbon dioxide, water vapor and ammonia are constantly vented with the exhaust air flow, while the temperature and adquate oxygen supply as well as the desired vapor concentration are maintained. The present procedure allows the animal to stay inside the chamber for an extended period of time and to perform the behavioral schedule while being exposed to an inhalant. Furthermore, the animal can be easily viewed through the glass wall of the chamber during behavioral performance in contrast to stainless steel cannister or pressure cooker used by others [1,11].

The present study indicates that xylene depresses the reinforcement rate in an operant schedule (fixed-ratio), and this depression can be manifested even at as low as 113 ppm concentration. Moser *et al.* [13] reported a biphasic effect on the DRL responding with an increase at 1400–2700 ppm and a decrease at 7000 ppm of xylene in mice. Furthermore, xylene produced a time-dependent biphasic change of FI responding in rats but the direction of this change was a function of concentration [9]. Such variations in xylene effects may be due to differences in the behavioral schedules, species of animal used and timing of behavioral measurement in relation to xylene exposure.

In the range finding experiment xylene caused a concentration-dependent decrease in the lever pressing rate at the 1st, 3rd, and 5th hours of exposure. Data at 2nd, 4th and 6th hours of this study indicate the development of acute tolerance. Since the behavioral measurement was started after 45 min of exposure in experiment I and after 15 min of exposure in experiment II, behavioral effects of xylene during the initial period of exposure are not presented. Furthermore, since xylene was infused at three successive concentrations in the range finding experiments, the observed depressant effect or tolerance might be influenced by immediate previous exposure to the lower xylene concentration. To determine the separate effect of each of the three concentrations, the two-hour study was designed. This experiment showed that within 15–30 min of exposure to xylene at three different concentrations there was no depression, while a prominent decrease of response was noted in the 45–60 min period. Both data from the 2nd hr of the two-hour experiment confirm the development of acute tolerance found in the range-finding experiment.

Exposure to 113 ppm of xylene was shown to decrease the reinforcement rate. However, exposure to a slightly lower concentration (e.g., 98.5±2.5 ppm) during five-hour sessions failed to produce any significant decrease. Thus the MEC for xylene appears to be 113 ppm, although a value between 98.5 ppm and 113 ppm cannot be excluded.

The transient disruption of behavior, as shown in this study, may be effected through the CNS action of xylene, or by its odor and irritant properties. The time required to produce the effect (e.g., 45–60 min of exposure) and lack of any effect during 15–30 min of exposure indicate that the observed behavioral effects are probably not due to irritant properties of xylene. Moreover, no observable signs of eye or nose irritation were noted during the exposure. Furthermore, during exposure to xylene, respiratory rate in mice was shown to remain unchanged at 460 ppm [3], whereas changes of EEG pattern in man indicating lowered vigilance were evident at 90–200 ppm [21].

The range finding experiment showed a concentration-dependent effect during xylene inhalation. A similar trend was reflected in the 2-hr study where the reinforcement rate decreased gradually with concentration during the 45–60 min period as well as during the 2nd hour (75–90 min and 105–120 min periods). However, the decrease of the reinforcement rate during 45–60 min period at 446 ppm, although significant, was not proportional.

This study also showed development of tolerance during the 2nd hour of xylene exposure. Savolainen *et al.* [22] noted a tolerance to xylene's effect on the equilibrium and the reaction time performance on human subjects after five successive exposure days. The acute tolerance observed in this study cannot be explained by induction of hepatic and renal microsomal enzyme systems [5,6], since it takes 1–2 weeks of exposure (6-hr/day) for such changes. The mechanism of development of this acute tolerance cannot be explained from the present study.

Thus the present study shows that inhalation of xylene vapor above 113 pm causes a transient decrease of reinforcement rate at hour 1 of exposure and acute tolerance seems to develop during hour 2 of exposure.

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