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Repeated inhalation of toluene by rats performing a signal detection task leads to behavioral tolerance on some performance measures ☆

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Abstract

Previous work showed that trichloroethylene (TCE) impairs sustained attention as evidenced by a reduction in accuracy and elevation of response latencies in rats trained to perform a visual signal detection task (SDT). This work also showed that these effects abate during repeated exposures if rats inhale TCE while performing the SDT. The present experiment sought to determine whether toluene, another commonly-used solvent, would induce tolerance similarly if inhaled repeatedly during SDT testing. Sixteen male, Long-Evans rats were trained to perform the SDT. Upon completion of training, rats were divided into 2 groups. In Phase I, concentration-effect functions were determined for toluene (0, 1200, 1600, 2000, 2400 ppm) in both groups. Toluene reduced the proportion of correct responses [*P*(correct)], and increased response time (RT) and response failures. In Phase II, Group-Tol inhaled 1600 ppm toluene while Group-Air inhaled clean air during 11 daily SDT sessions. In Group-Tol the effect of toluene on *P*(correct) abated after 3 days, while RT remained elevated for the duration of the repeated exposures. In Phase III, toluene concentration-effect functions were re-determined for both groups. Group-Air remained impaired on all test measures, whereas for Group-Tol, toluene did not reduce *P*(correct), but continued to increase RT. These data confirm our previous hypothesis that animals can develop tolerance to chemical exposures that impair appetitively-motivated behaviors if that impairment leads to loss of reinforcement.

Keywords: Attention; Organic solvent; Rat; Signal detection; Tolerance; Toluene

1. Introduction

Toluene is a clear, colorless liquid used in the processes of making gasoline and other fuels as well as an ingredient in paints, thinners, cleaners, nail polishes, adhesives and a variety of other household products. Inhalation is one of the main routes of exposure to toluene because of its volatile characteristic and widespread use in industry, households, and transportation vehicles. Consequently, it and other volatile organic compounds (VOCs) constitute a major fraction of the airborne pollutants with neurotoxic potential and pose a potentially significant hazard to health [16]. Additionally, many of these compounds are abused because of their similarities in physiological effect and modes of action to other drugs of abuse [2,19]. Thus, it is imperative that the neurotoxic effects of such compounds be well understood and characterized for a range of exposure scenarios.

Whereas much is known about the acute effects of toluene and other VOCs, questions remain about how an individual's sensitivity to these acute effects may change because of repeated exposure to the VOC. Specifically, with repeated exposure, acute responses may either decrease (tolerance), increase (sensitization), or remain the same. Further, a change in

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the sensitivity of an individual to a VOC means effectively that its potency has changed for that individual, and this difference in potency can be observed as a horizontal shift in the concentration-effect function that relates the inhaled concentration of the VOC to its effect.

Experimental studies have yielded mixed results concerning changes in sensitivity to the acute effects of toluene and other VOCs after repeated exposure. In previous reports on the behavioral effects of trichloroethylene (TCE) in rats, we showed that although performance on a signal detection task was impaired by the VOC in a concentration-related manner [6], these effects abated when inhalation exposures of 2000 ppm TCE were paired with task performance in daily 1-h test sessions [8,32]. In these latter two studies, tolerance developed in animals that performed the task daily during exposure, but not in animals receiving an equivalent daily TCE exposure after performing the task. We also discussed some of the factors that affect changes in sensitivity to these compounds, including the exposure conditions, the behaviors measured, the reinforcement contingencies in effect, and the time at which the behavioral measures were taken (*i.e.*, during or after exposure) [32]. We concluded that the development of tolerance was driven primarily by loss of reinforcement resulting from disruption of behavior associated with acute intoxication.

The current study was designed to assess whether repeated exposure to toluene alters the acute potency of toluene on signal detection behavior. Due to similarities in the pharmacological properties of TCE and toluene [2,17] and similarities in the effects of these solvents on performance of the SDT [6,10], we hypothesized that rats would become tolerant to the effects of toluene if they were exposed while performing the SDT. Instead of comparing exposures during and after behavioral testing, we designed this study to determine whether the potency of toluene on SDT performance would be reduced by repeated exposure to a behaviorally effective concentration of toluene. Specifically, we hypothesized that the concentration-effect functions (CEFs) for toluene on SDT behaviors would shift to the right in rats exposed to toluene during daily, 1-h test sessions.

To address this question, rats were trained to perform the SDT and were then divided into 2 groups with equivalent performance on the task. In Phase I of the study a CEF was determined for each group (CEF 1). In Phase II, Group-Tol was exposed to toluene (1600 ppm) and Group-Air was exposed to air daily for 11 days during the 1-h SDT test sessions. In Phase III, the toluene CEFs were re-determined for both groups (CEF 2). A rightward shift in the CEFs for toluene in Group-Tol would indicate that tolerance had developed.

2. Methods

2.1. Subjects

Sixteen male Long-Evans rats (Charles River, Portage, MI) were housed individually in suspended polycarbonate cages on heat-treated pine shavings in a housing facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) according to NIH guidelines. This animal research protocol was reviewed and approved by the NHEERL Institutional Animal Care and Use Committee which ensures conformance with the 1996 NRC "Guide for the Care and Use of Laboratory Animals", the Animal Welfare Act and Public Health Service Policy on the Humane Care and Use of Laboratory Animals. Lighting followed a L:D 12 h:12 h (0600:1800) photoperiod; all behavioral testing occurred in the light phase of the cycle. Each animal was maintained at 350±10 g body weight by scheduled home cage feeding (Ralston Purina, St. Louis, MO) after daily test sessions [1]; tap water was available *ad libitum* in the home cage. Rats were 4 months old at the start of training and 15 months old at the beginning of the toluene exposures.

2.2. Apparatus — operant behavior

Four 32.9-L operant-inhalation chambers were constructed of stainless steel and glass for the assessment of operant performance of rats inhaling controlled concentrations of solvent vapors [6,10]. The front wall of each of these chambers contained two retractable omnidirectional response levers; a food cup with a hinged, clear plastic door, centered between the levers; a house light; a signal light; and a 5-cm cone loudspeaker. The house and signal lights were mounted 15 cm above the floor of the chamber: the signal light was centered above the food cup, between the house light and the loudspeaker. Background white noise of 65 dB was generated in each chamber. Experimenter access and rat placement into the chamber was accomplished by removal of a transparent rear panel.

Signal stimuli were generated with the incandescent signal lamp (bulb #3019) by amplifying current from a 256-step digital-to-analog converter (DAC; Model L65-28, Coulbourn Instruments, Lehigh Valley, PA). Each lamp was adjusted to 1.66 lx with no attenuation as measured with a photometer (Model 450, EG and G, Inc., Salem MA) mounted on the glass door of the test chamber. The brightness of the signal light was varied by adjusting the attenuation of the DAC. Rats were presented with 4 signal intensities ranging from 0.09 to 1.66 lux. Each of the intensities was presented quasi-randomly during each test session against a background illumination level from the house light of 0.11 lx. Signals were generated using SKED-11 software (State Systems, Kalamazoo, MI) running on a PDP11/70 computer (Digital Equipment, Maynard, MA). The same software controlled vapor concentration monitoring and behavioral testing (see below).

2.3. Apparatus — toluene exposures

Toluene (99.5% spectrophotometric grade, Sigma-Aldrich Chemical Co., St. Louis, MO) vapor was generated within each operant-inhalation chamber using a modification of the method previously described [6,8]. Liquid toluene was dispensed into four heated, independent streams of nitrogen gas from glass syringes (SGE Incorporated, Apple Valley, MN) driven by programmable pumps (Model PHD 2000, Harvard Apparatus, Holliston, MA). Inlet air was cleaned with carbon and HEPA filters and conditioned to 22±2 °C and 50±10% relative

humidity; air flow rates through these exposure chambers were approximately 18 L/min. Toluene vapor was generated in the chambers with a heated J-tube using zero-grade N_2 as a sweep gas, and diluted into the incoming air stream to achieve the appropriate exposure concentration [26]. The rise time (t_{95}) of toluene vapor concentrations was 6 min; test sessions began 10 min after the start of exposures.

Toluene vapor concentrations were monitored in each chamber using infrared spectrophotometry (MIRAN 1A: The Foxboro Co., East Bridgewater, MA). The spectrophotometer was calibrated before each exposure phase began using a static, re-circulating closed-loop method. Toluene vapor concentrations in each operant-inhalation chamber were sampled sequentially in 5-min periods as previously described [6,10], yielding 2–3 samples per chamber per session. The vapor flow was manually adjusted by changing the flow rate of the syringe pump to maintain or regain the target concentration. Each rat was always tested and exposed in the same chamber. Target vapor concentrations (0, 1200, 1600, 2000, and 2400 ppm) included the nominal toluene concentration ±10%.

2.4. Signal detection task (SDT)

All rats were trained to perform a visual signal detection task as previously described [6,9]. Briefly, after a variable inter-trial interval, rats were required to report the occurrence or non-occurrence of a signal stimulus (a 300-ms light flash) by pressing one of two response levers. Both levers were inserted a variable (2–4 s) time after the signal period during each trial, and retracted when either was pressed. Signal and blank trials were intermixed unpredictably in equal number during each test session, and differed only in that no signal occurred during the signal period in a blank trial. One lever was designated as the signal lever and the other lever as the blank lever. Each correct response, i.e., a press on the signal lever on a signal trial or a press on the blank lever on a blank trial, caused illumination of the food cup light (2 s) and delivery of a food pellet (PJ Noyes Co., Lancaster, NH) on 80% of these trials. After each incorrect response (i.e. a press on the signal lever on a blank trial or a press on the blank lever on a signal trial), or after a response failure, the house light was turned off for 3 s and no food was delivered. Both levers were retracted when either lever was pressed or the rat failed to respond within 5 s (response failure). The task was conducted in daily test sessions at 5 trials per minute (approximately 300 trials per session). Each 60-min test session was preceded by a 10-min pretest interval, to allow the toluene vapor concentration to rise to its target level in the air. To reduce somnolence during the pretest interval, food pellets were made available during three 1-min periods, signaled by illumination of the food cup light, on a progressive ratio schedule of openings of the food cup door.

2.5. Behavior measurements

The number of "hits" (signal lever presses on signal trials), "correct rejections" (blank lever presses on blank trials), "false alarms" (signal lever presses on blank trials), and "misses" (blank lever presses on signal trials) were recorded for each 60 min test session. Similarly, response time (RT) was measured for each response type as the time between insertion of the levers into the chamber and the time at which a lever press was recorded. Toluene exposure induced a bias towards one lever or the other (blank or signal lever) that differed across animals. This inconsistent bias obviated the use of *P*(hit) and *P*(fa) to describe the effect of toluene on performance. Thus, the proportion of correct responses, *P*(correct), and overall response time, RT, were used as the primary dependent measures. These measures were defined as follows: *P*(correct)=(number of hits+number of correct rejections)/(numbers of hits+misses+correct rejections+false alarms); RT=(cRT for hits+cRT for misses+cRT for correct rejections+cRT for false alarms)/(number of hits+number of misses+number of correct rejections+number of false alarms), where cRT=the cumulative response time for all responses of each type in a test session. Finally, the number of response failures was recorded during each test session. Trials lacking a response were not repeated. *P*(correct) and RT data were used only for those rats that completed more than 150 trials in each test session.

2.6. Experimental design

2.6.1. Groups

Once trained, rats were divided into two groups (Group-Air and Group-Tol, $n=8$ per group) with equivalent accuracies and response times as determined by performance during 4 consecutive, daily SDT sessions immediately prior to the start of the toluene exposures.

2.6.2. Toluene exposures (Phases I, II and III)

The toluene exposures for each phase of the experiment are diagrammed in Table 1. In Phase I and Phase III, acute concentration-effect functions (CEFs 1 and 2) for toluene (0, 1200, 1600, 2000, and 2400 ppm) were determined in both groups (Group-Air and Group-Tol). In these phases, each concentration of toluene was given to each rat in both groups in a quasi-random order, balanced by group and operant chamber. One concentration was given to each rat per day (Mon–Fri), until each rat had received each concentration once, during each of these test phases. During the Phase II exposures, Group-Tol rats inhaled toluene (1600 ppm) while Group-Air inhaled air (0 ppm) during daily SDT test sessions. In this phase, toluene and air exposures occurred in consecutive, daily sessions (Mon–Fri) for 11 SDT test sessions.

Table 1
Experimental design

Baseline (week 0)	Groups	Phase I (weeks 1 to 4)	Phase II (weeks 5 and 6)	Phase III (week 7)
All rats $n=16$	Group-Air $n=8$	SDT+toluene (CEF 1)	SDT+air (0 ppm)	SDT+toluene (CEF 2)
	Group-Tol $n=8$	SDT+toluene (CEF 1)	SDT+toluene (1600 ppm)	SDT+toluene (CEF 2)

In Phase I, both groups inhaled toluene (0, 1200, 1600, 2000, and 2400 ppm) for the first concentration-effect function determination (CEF 1). In Phase II, Group-Air inhaled air only (0 ppm) while Group-Tol inhaled toluene (1600 ppm) during 11 daily SDT sessions. In Phase III, both groups again inhaled toluene (0, 1200, 1600, 2000, and 2400 ppm) for the second CEF determination (CEF 2).

2.6.3. Experimental timeline

Due to equipment failure during Phase I, the first set of acute exposures required 4 weeks to complete. The repeated exposures (Phase II) began 4 days after the completion of Phase I and continued for 11 days. These 11 days were interrupted by weekends and a holiday. This phase ended on a Friday. The second acute exposure series (Phase III) began 3 days later on a Monday and ended that Friday. Seven weeks were required to complete all exposures (see Table 1).

2.7. Data analysis

The factors critical to the hypothesis are differences between the acute concentration-effect functions (CEFs) derived from Phase I (CEF 1) and Phase III (CEF 2) within each group. *P*(correct), RT and response failures for each group were subject to independent analysis of variance (ANOVA) with Toluene concentration (0, 1200, 1600, 2000 and 2400 ppm) and Phase (I and III) as repeated measures [34]. Significant Toluene by Phase interactions indicated that the effect of toluene on a group's performance differed from Phase I to Phase III; step-down contrasts were then examined to determine the concentrations at which these differences occurred. A significant effect of Toluene concentration in the absence of a Toluene by Phase interaction indicated that the group's performance did not differ from Phase I to Phase III. In these cases the effects of toluene at each concentration are reported for the group collapsed across both phases.

Characterization of performance during the Phase II exposures is also important to the current hypothesis. Thus, *P*(correct) and RT were subject to independent ANOVAs with Group as a between-group factor and Day (1–11) as a repeated measure [34]. Changes in *P*(correct) and RT during the repeated exposures in Phase II were also assessed against pre-toluene baseline measures for each group derived prior to the start of the toluene exposures in Phase I. Response failures were not analyzed for Phase II.

Huynh-Feldt degree-of-freedom (*df*) corrections were used to minimize the effects of asymmetrical variance–covariance matrices with all repeated measures. The Type I error rate was set at 0.025 for the CEF comparisons (Phases I and III) to account for the separate analyses for each group and at 0.05 for the Phase II repeated exposures.

3. Results

3.1. Toluene exposures

One rat was eliminated from all data due to an insufficient number of acceptable toluene exposures, and 1 rat died due to unknown causes during Phase II. Thus, the number of rats used in the analysis fell to 14 ($n=7$ per group).

Results of the toluene exposures for these 14 rats are summarized in Table 2. Of the 148 individual measurements of the four toluene concentrations during Phase I, 11 were more than 10% below the nominal concentration. These samples altered the average concentration of two rats, one each at 2000 and 2400 ppm, to between 10% and 15% of the nominal concentration. Of the 188 individual measurements of the 1600 ppm concentration during Phase II, 1 sample fell between 10% and 15% of the target value. Finally, of the 149 individual measurements of the four toluene concentrations during Phase III, 1 sample fell 10% to 15% below the nominal concentration.

Table 2
Toluene concentrations during Phases I, II, and III

Nominal	Phase I				Phase II				Phase III			
	Actual	N	H	L	Actual	N	H	L	Actual	N	H	L
1200	1198±52	30	0	1	–	–	–	–	1186±37	31	0	0
1600	1546±73	34	0	2	1576±54	188	1	0	1588±40	35	0	1
2000	1907±78	42	0	4	–	–	–	–	2012±54	41	0	0
2400	2349±131	42	0	4	–	–	–	–	2467±75	42	0	0

Concentrations of toluene in the chamber air during exposure. Actual values are mean (±SD) ppm across the 14 rats and 2–3 determinations per exposure; n=number of measurements per concentration. The target range for each exposure was ±10% of the nominal concentration; the number of measurements that fell above (H) or below (L) this target range is indicated.

3.2. Phases I and III (CEF 1 vs. CEF 2)

Of the 14 rats with acceptable exposures, all performed at least 150 trials during the toluene exposures and thus were included in the *P*(correct) and RT analysis for the Phase I and Phase III comparisons. The minimum number of trials completed was 159 and occurred during Phase I (CEF 1) at the 2400 ppm concentration. All rats ate all pellets earned during the task and no residual effects of the toluene exposures were seen in subsequent SDT testing in air.

3.2.1. Group-Air

Toluene significantly affected *P*(correct), RT, and response failures in a concentration-related manner. These effects were equivalent for Group-Air in both Phases [all toluene by Phase interaction Ps>0.289]. Toluene reduced *P*(correct) (Fig. 1A) [effect of toluene: $F(4,24)=12.98$, $\varepsilon=0.91$, $P<0.0001$] with significant effects occurring during exposure to toluene at 1600, 2000 and 2400 ppm [all P's<0.0098]. RT was elevated by toluene (Fig. 1C) [effect of toluene: $F(4,24)=31.99$, $\varepsilon=0.52$, $P<0.0001$] at each concentration [all P's<0.0005]. Response failures (Fig. 1E) were also elevated [effect of toluene: $F(4,24)=21.22$, $\varepsilon=0.66$, $P<0.0001$] with significance reached during exposure to 2000 and 2400 ppm toluene [all P's<0.0086].

3.2.2. Group-Tol

Toluene significantly affected *P*(correct) and RT but not response failures in Group-Tol; these effects were not equivalent for Phase I and Phase III for each measure. *P*(correct) (Fig. 1B) was affected differently in the two phases [toluene by Phase interaction: $F(4,24)=7.95$, $\varepsilon=1.29$, $P<0.0003$]. Contrast analysis revealed that the CEFs differed in response to the 1600 [$F(1,6)=17.38$, $P<0.0059$], 2000 [$F(1,6)=8.80$, $P<0.025$] and 2400 ppm [$F(1,6)=20.26$, $P<0.0041$] concentrations. This difference was expressed as a rightward shift in the Phase III CEF, indicating tolerance had developed on *P*(correct). RT (Fig. 1D) was elevated similarly in Phase I and Phase III [toluene by Phase interaction: $P=0.62$] with significant effects of toluene [$F(4,24)=0.0001$,

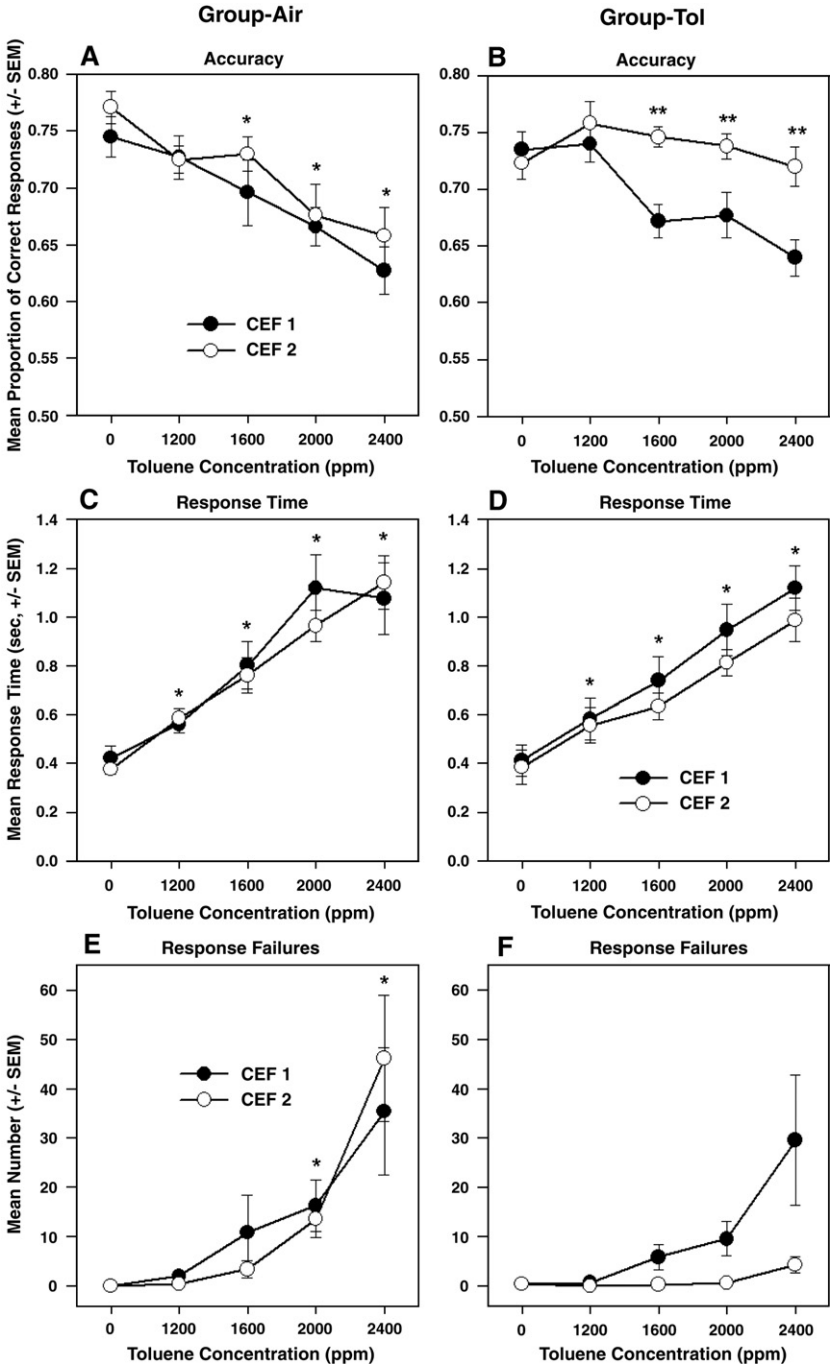

Fig. 1. Accuracy, response time, and response failures as a function of toluene concentration and phase for Group-Air (left panels) and Group-Tol (right panels). Each graph displays the toluene concentration-effect functions during Phase I (CEF 1, filled circles) and Phase III (CEF 2, open circles), for each measure. Single asterisks (*) indicate a significant effect of toluene at a given concentration for the combined CEF functions and no toluene by Phase interaction. Double asterisks (**) indicate a significant toluene by Phase interaction for the group, and where those differences were evident in the contrast analysis. Group-Air experienced equivalent impairments in accuracy (Fig. 1A), response time (Fig. 1C), and response failures (Fig. 1E) as a result of toluene exposure and no differences in effect were evident between the two CEF determinations. In contrast, Group-Tol showed significant improvement in accuracy measures (Fig. 1B) as a function of toluene during CEF 2 compared to CEF 1. Response Times (Fig. 1D) in Group-Tol were impaired equivalently in both phases. No significant effect of toluene or toluene by Phase interaction was seen with response failures in Group-Tol (Fig. 1F).

$\varepsilon = 0.53$, $P < 0.0001$] occurring at each concentration [all Ps < 0.0036]. Toluene did not significantly elevate response failures (Fig. 1F) [$P = 0.0503$], nor was there a difference between the 2 phases [toluene by Phase interaction: $P = 0.11$]. The lack of significance in the toluene by Phase interaction is probably due to the large variability in this measure among the rats.

3.3. Phase II: repeated exposures

As expected, significant differences in performance were evident between the two groups during the daily tests with exposures to toluene (Group-Tol) or air (Group-Air). Compared to baseline, toluene reduced P(correct) in Group-Tol (Fig. 2A) [Day

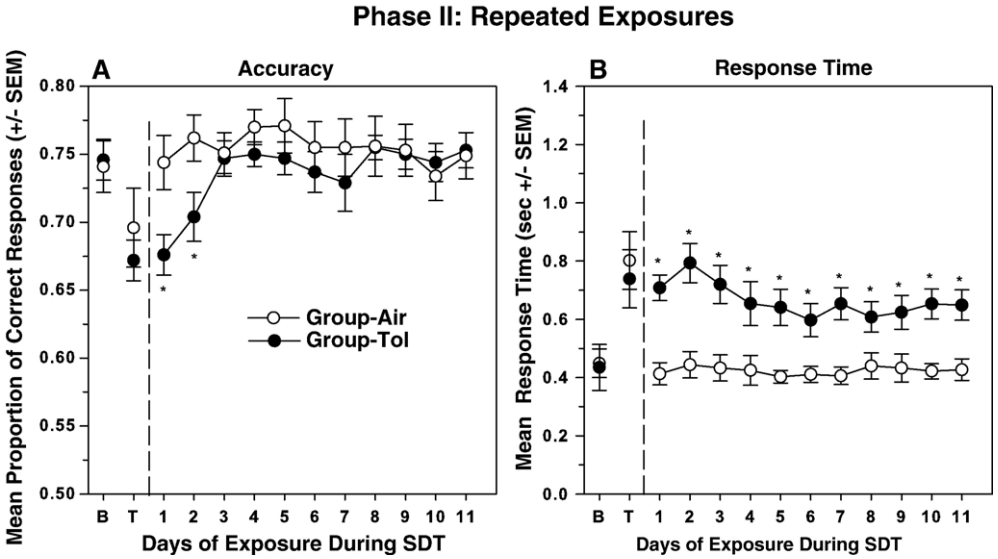

Fig. 2. Accuracy (Fig. 2A) and response time (Fig. 2B) during the repeated exposures in Phase II. Group-Air breathed air (0 ppm) while Group-Tol breathed toluene (1600 ppm). Pre-toluene baseline performance (*B*) and performance during the 1600 ppm concentration (*T*) of toluene during Phase I are shown for each group. Single asterisks (*) indicate significant differences between the performances of the two groups on a given day during the 11 daily exposures. Accuracy was reduced in Group-Tol on Day 1 and 2 but was not different from baseline (B) or Group-Air on Day 3 through 11 of the 11-day repeated toluene exposures. Response time was elevated in Group-Tol on each day of the toluene exposures compared to B and differed from Group-Air on each of these days.

by Group interaction: $F(11,132)=3.12$, $\varepsilon=0.69$, $P<0.0043$] on Day 1 [$F(1,12)=5.70$, $P<0.0343$] and Day 2 [$F(1,12)=5.35$, $P<0.0393$] of the 11 daily repeated exposures in Phase II. Univariate analyses revealed that *P*(correct) also differed between the two groups on these days [both *P*'s <0.0370] but were not different on Day 3–Day 11 (all *P*'s >0.2447). Toluene also elevated RT in Group-Tol during Phase II (Fig. 2B) [day by Group interaction: $F(11,132)=10.12$, $\varepsilon=0.50$, $P<0.0001$], which remained elevated above baseline on all 11 days of the repeated exposures [all *P*s <0.0047]. Univariate analyses confirmed differences in RT between the two groups on these days [all *P*'s <0.0299].

4. Discussion

These results show that rats develop tolerance to some but not all of the acute effects of inhaled toluene on performance of a visual signal detection task (SDT). Whereas both groups showed robust concentration-related impairments on three measures of task performance during Phase I (CEF 1), performance in Phase III (CEF 2), conducted after 11 days of performance of the task in toluene (Group-Tol) or air (Group-Air), showed a different pattern of response in the two groups. Compared to Phase 1, Group-Tol displayed marked improvement in *P*(correct) and a trend for fewer response failures during Phase III but no improvement in RT. This pattern indicates that tolerance had developed on the measure of accuracy, but not measures associated with response speed. Group-Air did not improve from Phase I to Phase III on any performance measure.

Further evidence for tolerance can be seen in the results of the repeated exposure phase of the study (Phase II). In that phase, Group-Tol became tolerant to 1600 ppm toluene by the third day of repeated exposure, as indicated by complete recovery from the initial toluene-induced deficit in *P*(correct), and partial recovery from the initial toluene-induced increase in RT (Fig. 2). It is interesting to note that the RT measures were longest (~0.8 s) on Day 2 of the exposures and then began to recover on Day 3, reaching stable levels by Day 4 (~0.6 s), where they stayed for the remainder of the 11-day exposure. This initial slowing of RT might reflect acquisition of an adaptation that allowed *P*(correct) to return to its baseline level. That is, restoring accuracy under the influence of toluene may have required the rats to slow their responding, in a form of speed-accuracy tradeoff. The relatively stable response time measures (Day 4 to Day 11) and the similarity in response time (~0.6–0.7 s) during the 1600 ppm toluene concentration in all three phases suggests that complete tolerance would not develop on RT if the exposure was carried out further.

These observations support our previous contention that tolerance to solvents is observed when the compound reduces the number of reinforcers received, and the subject is able to alter behavior to regain them [32]. In the current study, accuracy was reduced and response failures were increased by toluene, both of which reduced the number of reinforcers earned by the subject. Accuracy and response failures returned towards pre-toluene values during repeated toluene exposures in Phase II and were much less affected by toluene in Phase III. Thus these animals regained the reinforcers lost due to intoxication with toluene. On the other hand, reinforcement did not depend on RT unless the RT exceeded the limited hold period (5 s), causing a response failure. That is, as long as a response was made within the hold period, there was no cost for slow responding and therefore no incentive to regain baseline levels of response speed. The lack of improvement in RT is thus consistent with this mechanism for the development of tolerance.

Whether the tolerance observed here is strictly a behavioral adaptation or is facilitated by metabolic changes that either reduce toluene's uptake into the brain is uncertain, because we did not include a metabolic control condition in this study. However,

previous work with trichloroethylene (TCE) showed that tolerance developed only in the animals that performed the task in the presence of the solvent and not in animals that received the solvent after performing the task [8,32]. This tolerance was labeled "behavioral" due to its dependence on "intoxicated practice" [22] and was believed to be driven by the loss of food reinforcement due to poor performance during intoxication [20,32]. In a similar study, Rees et al. [33] tested rats under a fixed-consecutive-number schedule of reinforcement either before or after a 2-h exposure to toluene (1780–4500 ppm). Tolerance developed in some of the rats tested after exposure. No tolerance was observed in rats tested before exposure, when conditions were subsequently reversed and they were challenged with tests after exposure. These observations indicate that metabolic variables did not account for the tolerance that was observed in rats tested after exposure to toluene.

Behavioral tolerance has also been observed with drugs of abuse (e.g., ethanol [22,23]; benzodiazepines [3]; amphetamine [12,13,24,38]. In these studies, altered metabolism of the drug did not account for the tolerance that developed to the drugs. Also, studies on the effects of chronic amphetamine treatment and tolerance have repeatedly shown evidence of equivalent, if not elevated, brain amphetamine levels in animals repeatedly exposed [13].

In contrast, there is evidence that toluene and other solvents induce metabolizing enzymes in the liver [15,30] and lung [18], and these changes might alter the uptake, distribution and elimination of toluene in repeatedly exposed rats [37]. Since the acute effects of toluene and other VOCs are believed to depend upon the concentration of the compound in the brain at the time the effect is measured [4,5,27,28], reduced brain concentrations due to induced metabolism could explain the tolerance seen here. However, few studies have shown a connection between changes in metabolism and lowered blood or brain toluene concentrations. For example, blood toluene concentrations were equivalent after 1, 3 and 5 days of exposure to toluene (375 ppm) 6 h/day [18]. Another study found equivalent blood toluene concentrations after 1 and 18 days of repeated exposure to 1000 ppm toluene, 6 h/day [25]. In contrast, Stumph et al. [35] found a decrease in blood and brain toluene concentrations between the second (96.9 μg/ml and 200.72 μg/g, respectively) and third (78.8 μg/ml and 141.70 μg/g, respectively) weeks of repeated exposure to 2500 ppm toluene, 3 h/day, 7 d/week. Also, Elovaara et al. [15] found that brain toluene concentrations were decreased from 14.4 nmol/g after 1 week of exposure (300 pm, 6 h per day) to 11.2 nmol/g after 3 weeks of the same exposure. However, this decrease was not greater after 15 weeks exposure.

These latter two studies support the possibility that increased metabolism of toluene might lower blood and brain toluene concentrations when exposure is extended (3–6 h/day) and repeated over the course of several weeks (>2 weeks). It is unclear whether the exposure conditions of our experiment (1600 ppm × 70 min/day for 11 days during the repeated exposures) would induce metabolism of toluene such that blood and brain concentrations would be significantly reduced.

Kinetic studies of rats performing the SDT in our laboratory revealed that brain toluene concentrations rise during SDT test sessions from ~30 μg/g in the first 5 min to ~100 μg/g after 60 min of exposure to 2000 ppm toluene [unpublished observations]. Modeling of internal doses of toluene from the exposures used here suggests that deficits in behavior begin to appear at brain toluene concentrations of ~20 μg/g, and maximal impairment occurs at ~160 μg/g. Inhalation of 1600 ppm for 70 min yields an estimated concentration of ~105 μg/g in the brain. Thus enhanced metabolism of toluene would have to reduce this concentration to ~20 μg/g to account for the tolerance observed in this study. Given that the reductions previously observed in other studies from repeated exposure to toluene do not exceed 25%, it is unlikely that metabolic tolerance can account completely for the tolerance observed here. Nevertheless, a partial role for metabolism cannot be ruled out in this study.

It is also possible that habituation to the odor and minor irritation from inhaled toluene might contribute to the improvement in performance. Although toluene is not a potent irritant [31], its odor may disrupt the animal's behavior and could account for the poor performance seen in the initial acute study. However, if this were the case, one would expect a within-session tolerance to develop during the 60-min tests during CEF 1. This was not the case: accuracy and response times were increasingly impaired throughout the sessions during the acute challenges, in concert with increasing concentrations of toluene in the brain (data not shown) [6,10]. Thus, it is doubtful that habituation is the cause of tolerance seen in this study.

Although both TCE and toluene caused similar disruption on SDT performance (reduced accuracy and elevated RT), the accuracy decrement was expressed differently by the two compounds. That is, TCE reduced *P*(hit) (proportion of signals reported) and increased *P*(fa) (proportion of blank trials that were reported as signals), indicating that it did not induce a response bias (i.e., accuracy was reduced on both signal and blank trials) [8,32]. In contrast, toluene did not cause consistent changes in *P*(hit) and *P*(fa) across animals; thus the effects of toluene on accuracy were better described as an overall effect on *P*(correct) rather than as specific effects on *P*(hit) and *P*(fa).

Comparing the patterns of tolerance between toluene and TCE is complicated by differences in procedure. In contrast to the present study, rats in our earlier studies [8,32] were repeatedly exposed to TCE at either 2000 or 2400 ppm, and concentration-effect functions were not determined. Nevertheless, the patterns of tolerance in the two compounds were similar with tolerance developing to behavioral measures closely related to reinforcement when intoxicated practice occurred. The differences seen in the degree of tolerance between the two compounds may be due to differences in the pharmacokinetics and/or pharmacodynamics of the two compounds — for example, the rate of clearance from the body, metabolites produced and interactions of the VOCs with neuronal ion channels [11].

The robust concentration-related performance decrements caused by toluene are similar to effects of toluene on auditory signal detection [10] and effects of TCE on visual signal detection [6] in rats. Impairments in these measures are consistent with impaired sustained attention [7], which has also been reported to occur in humans acutely inhaling toluene [14]. Some of the mixed results found in the literature with regard to tolerance to toluene [21,29,33,36] can be explained by toluene-induced loss of

reinforcement (or lack thereof) [32]. One must appeal to obvious differences in study design (e.g., behavioral measures, species, toluene concentration, and exposure methods) to account for others. The tolerance seen here after repeated exposures complicates risk assessment of these VOCs, in light of the fact that most public exposures are repeated, whereas most experimental studies involve only acute exposures. Further studies are needed to assess the roles of metabolic and behavioral variables in the development of tolerance to toluene, as well as the degree to which this tolerance occurs in response to other VOCs.

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References

[1] J.S. Ali, V.B. Olszyk, D.D. Dunn, K.A. Lee, S.M. Kendall, R.R. Rhoderick, P.J. Bushnell, A Lotus 1-2-3-based system for recording and maintaining body weight of laboratory animals, Behav. Res. Meth. Instrum. Comput. 24 (1992) 82–87.
[2] R.L. Balster, Neural basis of inhalant abuse, Drug Alcohol Depend. 51 (1998) 207–214.
[3] S.E. Bowen, S.C. Fowler, J.A. Stanford, M.J. Kallman, Behavioral tolerance to the force differentiation effects of diazepam and midazolam in rats, Psychopharmacology 148 (4) (2000) 327–335.
[4] W.K. Boyes, P.J. Bushnell, K.M. Crofton, M.V. Evans, J.E. Simmons, Neurotoxic and pharmacokinetic responses to trichloroethylene as a function of exposure scenario, Environ. Health Perspect 108 (Suppl 2) (2000) 317–322.
[5] W.K. Boyes, M.V. Evans, C. Eklund, P. Janssen, J.E. Simmons, Duration adjustment of acute exposure guideline level values for trichloroethylene using a physiologically-based pharmacokinetic model, Risk Anal. 25 (3) (2005) 677–686.
[6] P.J. Bushnell, Concentration-time relationships for the effects of inhaled trichloroethylene on detection of visual signals in rats, Fundam. Appl. Toxicol. 36 (1997) 30–38.
[7] P.J. Bushnell, Detection of visual signals by rats: effects of signal intensity, event rate and task type, Behav. Processes 46 (1999) 141–150.
[8] P.J. Bushnell, W.M. Oshiro, Behavioral components of tolerance to repeated inhalation of trichloroethylene (TCE) in rats, Neurotoxicol. Teratol. 22 (2000) 221–229.
[9] P.J. Bushnell, D.C. Rice, Behavioral assessments of learning and attention rats exposed perinatally to 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126), Neurotoxicol. Teratol. 21 (1999) 381–392.
[10] P.J. Bushnell, K.L. Kelly, K.M. Crofton, Effects of toluene inhalation on detection of auditory signals in rats, Neurotoxicol. Teratol. 16 (1994) 149–160.
[11] P.J. Bushnell, T.J. Shafer, A.S. Bale, W.K. Boyes, Development and applications of an exposure-dose-response model for organic solvents, Environ. Toxicol. Pharmacol. 19 (2005) 607–614.
[12] C. Demellweek, A.J. Goudie, An analysis of behavioural mechanisms involved in the acquisition of amphetamine anorectic tolerance, Psychopharmacology (Berl) 79 (1983) 58–66.
[13] C. Demellweek, A.J. Goudie, Behavioural tolerance to amphetamine and other psychostimulants: the case for considering behavioural mechanisms, Psychopharmacology 80 (1983) 287–307.
[14] R.B. Dick, J.V. Setzer, R. Wart, M.B. Hayden, B.J. Taylor, B. Tolos, V. Putz-Anderson, Effects of acute exposure to toluene and methyleketone on psychomotor performance, Int. Arch. Occup. Environ. Health 43 (1984) 91–109.
[15] E. Elovaara, H. Savolainen, P. Pfäffli, H. Vainio, Effects of subacute toluene inhalation on its metabolism and disposition in rat, Arch. Toxicol. (Suppl 2) (1979) 345–348.
[16] EPA, 1997 Update to ORD's Strategic Plan, Office of Research and Development, U.S. EPA, Report # EPA/600/R-97/015, 1997.
[17] E.B. Evans, R.L. Balster, CNS depressant effects of volatile organic solvents, Neurosci. Biobehav. Rev. 15 (1991) 233–241.
[18] G.M. Furman, D.M. Silverman, R.A. Schatz, Inhibition of rat lung mixed-function oxidase activity following repeated low-level toluene inhalation: possible role of toluene metabolites, J. Toxicol. Environ. Health, Part A 54 (1998) 633–645.
[19] M.R. Gerasimov, R.A. Ferrieri, W.K. Schiffer, J. Logan, S.J. Gatley, A.N. Gifford, D.A. Alexoff, D.A. Marsteller, C. Shea, V. Garza, P. Carter, P. King, C.R. Ashby, S. Vitkun, S.L. Dewey, Study of brain uptake and biodistribution of [11C] toluene in non-human primates and mice, Life Sci. 70 (23) (2002) 2811–2828.
[20] A.J. Goudie, C. Demellweek, Conditioning factors in drug tolerance, in: S.R. Goldberg, I.P. Stolerman (Eds.), Behavioral Analysis of the Drug Dependence, Academic Press, New York, 1986, pp. 225–286.
[21] D.J. Hinman, Tolerance and reverse tolerance to toluene inhalation: effects on open-field behavior, Pharmacol. Biochem. Behav. 21 (1984) 625–631.
[22] F.A. Holloway, D.C. Bird, J.A. Holloway, R.C. Michaelis, Behavioral factors in the development of tolerance to ethanol's effects, Pharmacol. Biochem. Behav. 29 (1988) 105–113.
[23] F.A. Holloway, R.C. Michaelis, R.D. Harland, J.R. Criado, D.V. Gauvin, Tolerance to ethanol's effects on operant performance in rats: role of number and pattern of intoxicated practice opportunities, Psychopharmacology 109 (1992) 112–120.
[24] K.M. Hughes, L. Popi, D.L Wolgin, Loss of tolerance to amphetamine-induced hypophagia in rats: homeostatic readjustment vs. instrumental learning, Pharmacol. Biochem. Behav. 64 (1) (1999) 177–182.
[25] T. Ikeda, N. Maehara, T. Sadamoto, I. Harabuchi, K. Yamamura, H. Miyake, Effects of toluene exposure on the rest-activity cycle of rats, Toxicol. Lett. 9 (1981) 255–265.
[26] R.R. Miller, R.L. Letts, W.J. Potts, M.J. McKenna, Improved methodology for generating controlled test atmospheres, Am. Ind. Hyg. Assoc. J. 41 (1980) 844–846.
[27] M. Miyagawa, T. Honma, M. Sato, H. Hasegawa, Effects of single exposure to toluene on operant behavior and brain toluene levels in rats, Ind. Health 22 (2) (1984) 127–131.
[28] M. Miyagawa, T. Honma, M. Sato, H. Hasegawa, Behavioral change after single exposure to toluene, and brain toluene levels in rats, Ind. Health 24 (1986) 157–161.
[29] V.C. Moser, R.L. Balster, The effects of acute and repeated toluene exposure on operant behavior in mice, Neurobehav. Toxicol. Teratol. (1981) 471–475.
[30] T. Nakajima, R. Wang, N. Murayama, Immunochemical assessment of the influence of nutritional, physiological and environmental factors on the metabolism of toluene, Int. Arch. Occup. Environ. Health 65 (1993) S127–S130.
[31] G. Nielsen, Y. Alarie, Sensory irritation, pulmonary irritation, and respiratory stimulation by airborne benzene and alkylbenzenes, Toxicol. Appl. Pharmacol. 65 (1982) 459–477.
[32] W.M. Oshiro, T.Q. Krantz, P.J. Bushnell, Characterizing tolerance to trichloroethylene (TCE): effects of repeated inhalation of TCE on performance of a signal detection task in rats, Neurotoxicol Teratol. 23 (2001) 617–628.
[33] D.C. Rees, R.W. Wood, V.G. Laties, Evidence of tolerance following repeated exposure to toluene in the rat, Pharmacol. Biochem. Behav. 32 (1989) 283–291.
[34] SAS, SAS/Stat. Version 6, 4th Edition, SAS Institute, Cary, NC, 1990.
[35] M.J. Stumph, F.W. Weir, M.W. Noall, Comparison of blood and brain toluene concentrations and circulating triglyceride levels resulting from acute and repeated exposures in rats, Am. Ind. Hyg. Assoc. J. 46 (5) (1985) 244–250.
[36] J.D. Taylor, H.L. Evans, Effects of toluene inhalation on behavior and expired carbon dioxide in macaque monkeys, Toxicol. Appl. Pharmacol. 80 (1985) 487–495.
[37] R. Toftgård, J.A. Gustafsson, Biotransformation of organic solvents, Scand. J. Work Environ. & Health 6 (1980) 1–18.
[38] D.L. Wolgin, G.B. Thompson, I.A. Oslan, Tolerance to amphetamine: contingent suppression of stereotypy mediates recovery of feeding, Behav. Neurosci. 101 (1987) 264–271.