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Abuse Pattern of Toluene Exposure Alters Mouse Behavior in a Waiting-for-Reward Operant Task

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Abstract

Inhaling solvents for recreational purposes continues to be a world-wide public health concern. Toluene, a volatile solvent in many abused products, adversely affects the central nervous system. However, the long-term neurobehavioral effects of exposure to high-concentration, binge patterns typical of toluene abuse remain understudied. We studied the behavioral effects of repeated toluene exposure on cognitive function following binge toluene exposure on behavioral impulse control in Swiss Webster mice using a “wait-for-reward” operant task. Mice were trained on a fixed-ratio (FR) schedule using sweetened milk as a reward. Upon achieving FR15, a wait component was added which delivered free rewards in the absence of responses at increasing time intervals (2 sec, 4 sec, 6 sec, etc...). Mice continued to receive free rewards until they pressed a lever that reinstated the FR component (FR Reset). Once proficient in the FR-Wait task, mice were exposed to either 1,000 ppm, 3,600 ppm or 6,000 ppm toluene, or 0 ppm (air controls) for 30 min per day for 40 days. To avoid acute effects of toluene exposure, behavior was assessed 23 hours later. Repeated toluene exposure decreased response rates, the number of FR resets, and increased mean wait time, resulting in a higher response-to-reinforcer ratio than exhibited by controls. Mice receiving the higher exposure level (6,000 ppm) showed a dramatic decrease in the number of rewards received, which was reversed when toluene exposure ceased. Mice receiving the lower exposure level (1,000 ppm) showed little change in the number of rewards. These results indicate that repeated binge exposures to high concentrations of toluene can significantly interfere with performance as measured by a waiting-for-reward task, suggesting a significant impact on cognitive and/or psychomotor function.

1.0 Introduction

Intentionally inhaling chemical fumes to achieve euphoric effects continues to be a popular method of drug abuse throughout the world [34] in part because of the ready availability of many products to “huff” and the uncomplicated methods of administration. Paint thinners, gasoline, nail polish remover and many commonly used household cleaning products are potential sources of abused chemicals. Exposure levels during episodes of abuse are much higher than concentrations present during typical occupational or incidental exposures to these same chemicals. For example, in contrast to an 8-hr exposure to ~100 ppm of toluene in a workplace, abuse typically can involve >20 deep inhalations of very high solvent concentrations (likely more than 5,000 ppm) over a very short period of time (10–15 min) [8,

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45]. Intoxication occurs in seconds and effects can last up to 60 min. Dose-dependent effects include disinhibition progressing to sedation, and euphoria, excitement, floating sensations, dizziness, slurred speech, ataxia and a sense of heightened power [12].

One troubling aspect of this problem is the young average age at which inhalant abuse begins. Nearly 6% of children in the United States have tried inhalants by the time they reach 4th grade, with almost 20% of 8th-graders saying that they had abused inhalants at least once in their lifetime [21]. In 2005, 877,000 individuals 12 years old or older used inhalants for the first time with 72.3 percent being under the age of 18 years at first use (average age was 16.1 years of age) [41,42]. Other recent trends from the National Survey on Drug Use and Health [41] show that inhalant abuse persists among adolescents and that an annual average of 4.5 % of youths aged 12 to 17 (approximately 1.1 million adolescents) used inhalants in the past year [41] and >600,000 boys and girls each year use inhalants for the first time. Within this growing trend of inhalant drug abuse in general, over 30% of these youths reported using inhalants containing toluene.

There are a growing number of reports showing that severe and irreversible brain damage can result from continued inhalant abuse. Dose-dependent effects of chronic solvent abuse include cerebellar damage [14,15,25,28], white matter abnormalities, (including MRI T2 hypo- or hyper-intensities in cerebrum, thalamus, basal ganglia and cerebellum) [22,44,48], and neuronal atrophy of hippocampus, corpus callosum and cerebellar vermis [11,35,36,40,48, 49]. Long-term inhalation of solvents, particularly toluene, is also associated with a number of severe neurological signs in people, including cerebellar ataxia, peripheral neuropathy, convulsions, and encephalopathy [13,20,23,28]. Chronic solvent exposure has also been shown to produce apathy and deficits in attention, memory and visuospatial function in humans [1, 20,27].

Given the increasing prevalence of inhalant abuse, the need for research into the cognitive and behavioral manifestations of chronic toluene-associated neural deficits is essential. Animal models of inhalant exposures are powerful tools for studying acute and chronic biobehavioral effects [6]. Depending upon the pattern of toluene exposure in rats and mice, there are reports of effects on motor function and activity (both hyperactivity and hypoactivity), as well as deficits in learning (for review see [6]). Toluene and other inhalants have also been shown to have reversible disruptive effects on operant response rates [3,17,18,30–33]. Some of these effects appear to be greater after binge patterns of abuse exposure than after lower-level patterns of toluene exposure [6]. Despite the differential effects following binge exposure, to our knowledge, there are no published studies assessing attention and/or higher cognitive processes in rodents after binge patterns of toluene exposure. In the current study, we assessed the effects of binge toluene exposure on behavior in the mouse using a “waiting-for-reward” operant task, a conditioning schedule of reinforcement used previously in young rats exposed to lead [7]. We hypothesized that mice exposed to toluene would have greater “impulsivity,” or a loss of inhibitory control and be less likely to inhibit responses during the free-reward component which would result in shorter wait times, a greater number of FR resets, and an overall decrease in response efficiency (i.e., more responses required for each reinforcement earned).

2.0 Materials and methods

2.1. Subjects

All animal procedures had prior approval by the Wayne State University Institutional Animal Care and Use Committee and were in accordance with the NIH “Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH Publication No. 85-23, revised 1996). Forty experimentally naïve male Swiss-Webster mice (CFW, Charles River Co., Portage, MI) were received at six weeks of age and

weighed an average of 29.8 g (± 0.54 sem) on arrival. Mice were housed individually in clear 18×29×13 cm plastic cages with wood chip bedding in a room maintained on a 12 L:12 D cycle (lights on at 0600 hrs) with temperature controlled to 20°C–22°C and relative humidity levels between 40% and 70%. Animals were weighed daily and maintained between 25–35 grams by a restricted diet of post-session feeding of 5–7 grams of rodent chow per day (Laboratory Rodent Chow, Ralston-Purina Co., St. Louis, MO). Water was provided *ad lib* while the mice were in their home cages with no access for 2–3 hours during experiments in the laboratory.

2.2. Apparatus

Behavioral training and testing were conducted in ten computer-interfaced operant conditioning chambers. Briefly, the operant chambers had stainless steel rod floors, three aluminum walls, and a fourth Plexiglas wall with a door. Each chamber was enclosed in a cubicle that attenuated external light and sound and was equipped with two response levers (Med Associates, Fairfax, VT, USA) that extended 0.8 cm into the operant conditioning chamber. A ventilation fan provided masking noise. A recessed food trough was located midway between the levers into which a dipper could deliver 3-sec access to 0.02 ml of evaporated milk. Finally, the chamber contained one houselight located near the ceiling at the rear of the chamber two stimulus lights that were located above each lever. Illumination of the houselight signaled that the session was in progress.

2.3. Operant Training Procedures

The current FR-Wait operant procedure was designed to measure waiting behavior and delays in reinforcement, which have been interpreted to reflect differences in inhibitory control – or “impulsivity” – and was based on an earlier experiment studying the effects of lead exposure in rats [7]. Briefly, animals were trained to respond on one lever under a fixed ratio (FR) schedule to obtain food pellets and then refrain from responding on a second lever to obtain “free” pellets with the time between “free” pellets increasing after each “free” pellet delivery. Once trained, an animal would emit a total of 15 lever press responses (FR 15) which produced food delivery and after earning the FR pellet, the animal could obtain “free” pellets at increasing intervals (2s, 4s, 6s, etc.) by inhibiting its responses on another lever, which reinstated the FR requirement.

In the present investigation, mice were initially trained to press the right lever in the presence of an illuminated stimulus light located above the lever for milk reinforcement. The milk reward consisted of sweetened condensed milk, water, and sugar in a 1:2:1 v/v/w ratio, which was prepared fresh daily. Mice were trained on a fixed ratio schedule (FR) five days a week until they became proficient at the task and reached FR15, that is, receiving reward for each 15 lever presses. Upon achieving FR15, a “Wait component” was added to the training (see Figure 1). After animals had successfully completed each FR15, the right lever was retracted and the light above the lever was extinguished. During the “Wait component”, the left lever was extended, the light above the lever was illuminated and “free” rewards were delivered. The mice continued to receive “free” rewards at increasingly longer time intervals that incremented by 2 seconds (i.e., 2 sec, 4 sec, 6 sec, etc.) until they pressed the left signaled lever which ended the “Wait component” and terminated the “free” rewards (left lever retracted). This reinstated the FR component (an FR Reset) with the right lever re-extended requiring the mouse to emit 15 lever presses on the right lever to receive their next reinforcer. Successful completion of 15 responses terminated the “FR component” and the mice re-entered the “Wait component” in which “free” rewards were once again available as described previously. There was no upper limit to the duration of the “Wait component” within the 30-min session, and the FR component was reset only with a response on the signaled left operant lever which the animal could do at any time during the “Wait component”. Once the animals were responding on the FR 15/“Wait

component” schedule, behavior was allowed to stabilize over 15 sessions. Animals were then randomly assigned to one of four exposure groups (N=10/group).

2.4. Inhalation exposure

Toluene vapor exposures were done in a static exposure system consisting of sealed 36-liter cylindrical glass jars with sealed acrylic lids (identical to the system detailed in [5]). The lids had injection ports, a fan and a stainless-steel-mesh box holding filter paper. During exposures, mice were placed onto a grid floor 20 cm from the bottom of the exposure chamber. For air-only, 0 ppm exposures, the lid was sealed, nothing injected onto the filter paper, and the fan turned on. For daily toluene exposures, the lid was sealed and a calculated amount of solvent (156 μ l for 1,000 ppm, 564 μ l for 3,600 ppm, and 939 μ l for 6,000 ppm) was injected onto filter paper suspended below the sealed lid. The fan was then turned on, which volatilized and distributed the toluene within the exposure chamber. In addition to the 0 ppm air control, the mice were exposed to one of three concentrations of toluene – 1,000 ppm, 3,600 ppm and 6,000 ppm. These concentrations were chosen based on known toluene values from previous behavioral studies [6].

Toluene (T-324, Fisher Scientific, Fairlawn, NJ, USA) was purchased commercially (purity $\geq$ 99.5%). Vapor concentrations were calculated nominal concentrations. Toluene vapor concentrations were confirmed by single wavelength-monitoring infrared spectrometry (Miran 1A, Foxboro Analytical). Mean concentrations of toluene were within 3% of nominal ~2.5 min after the solvent was added and remained within 2% of the nominal concentrations throughout the 30-min exposures. Levels of waste gases (i.e., water vapor and CO₂) had been previously monitored during pilot studies and changes during 15-min sessions with rats or mice were negligible.

2.5. Operant Testing Procedures after Toluene Exposure

Performance on the FR-Wait Task was tested for 40 days after exposures to toluene. To avoid acute effects of toluene exposure, operant behavior was assessed ~22 to 23 hours after each exposure. During this testing phase, animals continued to be exposed to toluene for 30 min a day for 40 days. After 40 days, the daily toluene exposure ceased. During this “recovery” phase, animals were exposed to air only (i.e., 0 ppm toluene) for 30 min a day for another 40 days, and performance on the FR-Wait Task continued as before.

2.6. Statistical Analysis

The mean times to wait for “free” milk reinforcement was derived as the average of the individual waiting times prior to each FR reset (i.e., the time a mouse would wait between “free” rewards before resetting the FR15 component; see Figure 1). As a measure of efficiency, the total number of responses in a session were divided by the total number of reinforcers earned in that session (in both the FR and Wait components) to yield “responses per reinforcement” measures. These measures, along with the number of “free” rewards and FR resets, were analyzed using repeated-measures analyses of variance (ANOVAs) in blocks of 4 sessions (i.e., 10 blocks for 40-day “exposure” period and 10 blocks for the 40-day “recovery” period with each block of 4 sessions reflecting the mean of the 4 sessions) with Toluene concentration serving as the between-groups factor and Blocks of days as the within-subjects factor. All significant ANOVAs were followed by Tukey’s *post hoc* comparisons as needed. A significance level of $\alpha = 0.05$ was maintained for all analyses.

3.0 Results

At the end of training on the FR-Wait schedule of reinforcement, the animals were receiving an average of 112.69 ($\pm$ 3.26 sem) free milk rewards, 34.14 ($\pm$ 2.66 sem) FR resets, waiting an

average of $65.28 (\pm 9.68 \text{ sem})$ seconds, and responding at $0.34 (\pm 0.02 \text{ sem})$ responses/sec per 30-min session over the last eight sessions. However, prior to any treatments, preliminary analyses of baseline data indicated significant differences among the different toluene exposure groups on several measures (see Table 1 for details). To standardize data for comparison of Toluene effects across Blocks during both the exposure and recovery periods, data were presented and analyzed as a percent of the pre-toluene exposure baseline mean of all animals for the last eight baseline days (2 blocks of 4 sessions each).

3.1. Weights

Toluene Exposure Period—As expected, there was significant weight gain during the exposure period in all groups, averaging 9 g (or 31 % of weight on the first day of exposure), $F(9,324) = 189.06$, $p < 0.0001$. There were no significant differences among groups ($p = 0.83$) but there was a significant Day by Group interaction, $F(27,324) = 6.03$, $p < 0.001$, which was due to decreases in weight gain for the 1,000 and 6,000 ppm toluene groups from exposure day 32 to day 40 and the 3,600 ppm toluene group from exposure day 36 to day 40 (data not shown).

Recovery Period—While there were no significant differences among groups ($p = 0.56$), there was a significant main effect for Day during the recovery period, $F(9,324) = 13.06$, $p < 0.0001$, and a significant Day by Group interaction, $F(27,324) = 2.39$, $p < 0.001$, which were due to the initial decreases in weight for all three toluene groups from recovery day 1 to day 20 (data not shown).

3.2. Free Rewards

Toluene Exposure Period—A significant main effect of Toluene exposure was seen for the number of free rewards obtained, $F(3,36) = 10.59$, $p < 0.001$. *Post hoc* analyses revealed that both the 3,600 ppm and 6,000 ppm groups earned significantly fewer free rewards during the Wait component than the 0 ppm and 1,000 ppm groups (p 's < 0.001). A significant main effect for Blocks, $F(9,324) = 6.68$, $p < 0.0001$, and a significant Block $\times$ Toluene interaction were found, $F(27,324) = 5.70$, $p < 0.0001$. This main effect and interaction were due to the decreases in free rewards during this Wait component for both the 3,600 ppm and 6,000 ppm groups from day 1 to day 20, and the increases in rewards received for the 3,600 ppm group during the last 20 days of toluene exposure (Figure 2, left panel).

Recovery Period—A significant main effect of Toluene, $F(3,36) = 6.10$, $p < 0.001$, was again observed with *post hoc* analyses revealing that both the 3,600 ppm ($p = 0.05$) and 6,000 ppm ($p < 0.01$) groups continued to earn significantly fewer rewards than the 0 ppm group. A significant main effect for Block, $F(9,324) = 4.52$, $p < 0.001$, and a significant Block $\times$ Toluene interaction were found, $F(27,324) = 1.88$, $p < 0.01$. This main effect and interaction were due to the increases in free rewards immediately following cessation of toluene for all groups and the continued failure of the 6,000 ppm group to earn as many rewards as the other groups did throughout the recovery period (Figure 2, right panel).

3.3. FR Resets

Toluene Exposure Period—As seen in Figure 3, Toluene exposure produced a significant effect on the total number of FR Resets, $F(3,36) = 12.03$, $p < 0.001$. *Post hoc* analysis showed that the 3,600 ppm and 6,000 ppm groups reset the FR component less frequently than the 0 ppm group (p 's < 0.01). A significant main effect was observed for Block, $F(9,324) = 3.30$, $p < 0.001$, along with a significant Block $\times$ Toluene interaction, $F(27,324) = 8.38$, $p < 0.001$. As was observed with the free reward measure, this main effect and interaction were due to the decreases in FR Resets for both the 3,600 ppm and 6,000 ppm groups from day 1 to day 20

and the subsequent increase in FR Resets for the 1,000 ppm and 3,600 ppm group during the last 20 days of toluene exposure.

Recovery Period—A significant main effect of prior Toluene exposure, $F(3,36)=5.64$, $p<0.01$, was still observed for FR Resets during recovery (Figure 3, right panel). *Post hoc* analyses revealed that the 1,000 ppm group had a higher number of FR Resets than the two higher concentration exposure groups ($p's<0.05$). However, none of the toluene-exposed groups differed significantly from the 0 ppm, air-exposed controls. There was no significant main effect for Day, ($p=0.17$), and the Block $\times$ Toluene interaction approached but did not reach significance, ($p=0.07$).

3.4. Mean Wait Time

Toluene Exposure Period—Toluene exposure significantly lengthened mean wait times, $F(3,36)=5.97$, $p<0.01$, with *post hoc* analysis, and the left side of Figure 4, showing clearly that the 6,000 ppm group had much longer mean wait times than all of the other groups ($p<0.01$), which did not differ significantly from each other. In addition, a significant main effect of Block was observed, $F(9,324)=5.25$, $p<0.001$, as well as a significant Toluene $\times$ Block interaction, $F(27,324)=3.93$, $p<0.001$, both also due to the significantly increased mean wait times for the 6,000 ppm group after 20 days of toluene exposure, and remained elevated for the remainder of the exposure period.

Recovery Period—There were no significant main effects for prior Toluene exposure ($p=0.90$) or Day ($p=0.45$), or their interaction ($p=0.30$) on the mean wait times during the post-toluene recovery.

3.5. Response Rate

Toluene Exposure Period—A significant main effect of Toluene exposure was observed for overall response rates, $F(3,36)=7.12$, $p<0.001$, with *post hoc* analyses supporting what is shown in Figure 5: both the 3,600 ppm and 6,000 ppm groups responded at significantly lower rates than the 0 ppm and 1,000 ppm groups ($p's<0.05$). A significant main effect for Blocks, $F(9, 324) = 4.23$, $p < 0.001$, and a significant Block $\times$ Toluene interaction was found, $F(27, 324) = 3.24$, $p < 0.0001$. This main effect and interaction were due to the decreases in responding from control levels for both the 3600 and 6000 ppm groups from day 1 to day 20 and the increases that occurred for the 3600 ppm group during the last 20 days of exposure (Figure 5).

Recovery Period—A significant main effect of Toluene, $F(3, 36) = 6.09$, $p < 0.001$, was again observed with *post hoc* analyses revealing that both the 3,600 ppm ($p = 0.05$) and 6,000 ppm groups ($p < 0.01$) continued to respond at lower rates than the 0 ppm group. While no significant main effect was observed for Blocks, $p > 0.11$, a significant Block $\times$ Toluene interaction was found, $F(27,324)=1.92$, $p<0.01$. This interaction was due to the increases in response rates of the 6,000 ppm group immediately following cessation of toluene and the continued increase that was observed for the 1,000 ppm group throughout the recovery period (Figure 5, right panel).

3.6. Responses per Reinforcement

Toluene Exposure Period—A significant main effect of Toluene exposure was also seen for the responses-per-reward ratio, $F(3,36)=7.01$, $p<0.001$ (Figure 6, left panel). *Post hoc* analyses revealed that the ratio for the 3,600 ppm toluene-exposed group was significantly higher (i.e., emitted more responses for each reward received) than the 0 ppm and 6,000 ppm groups ($p's < 0.05$). The 1,000 ppm toluene group also had a higher responses-per-reward ratio

than the 6,000 ppm group ($p < 0.05$). A significant main effect for Block, $F(9,324) = 5.96$, $p < 0.001$, and a Block $\times$ Toluene interaction were found, $F(27,324) = 5.14$, $p < 0.001$. These reflected the overall decreases in the ratio for all the toluene groups through day 20, a pattern which continued for the 6,000 ppm group for the remainder of the exposure period, plus a reversal for the 3,600 ppm group whose ratio increased during the last 20 days of exposure (Figure 6, left panel).

Recovery Period—As seen on the right panel of Figure 6, a significant main effect of prior Toluene exposure was observed during the recovery period, $F(3,36) = 11.11$, $p < 0.001$, with *post hoc* analyses revealing that the 3,600 ppm toluene-exposed group had a significantly higher responses-per-reward ratio than any of the other groups (p 's < 0.05). Neither the main effect of Block ($p = 0.25$) nor the Block $\times$ Toluene interaction were significant for the ratio during the recovery period ($p = 0.17$).

4.0 Discussion

In the current study, we tested the hypothesis that brief, repeated high-concentration exposures to toluene would produce behavioral deficits in mice. These results are particularly important because the binge pattern of toluene exposure that was used models patterns of exposure typical of solvent abuse. Behavior was measured in a “wait-for-reward” operant task designed to assess inhibitory control and responses to reinforcement delay [7]. While our results demonstrate that repeated exposure to toluene can significantly alter mouse behavior across several measures, we do not believe the patterns of behavioral change in this task mean that toluene increased “impulsivity” in the mice. Because our study design included a “washout period” of 22–23 hours after exposures in which toluene should not have been present in the body, these effects would not be expected to be due to any acute impact of toluene intoxication.

Interestingly, the manifestation of these persistent behavioral effects varied by toluene concentration. As illustrated in Figures 2 and 3, some evidence for changes in behavior consistent with response inhibition was demonstrated during exposure for the 1000 ppm toluene group in the current set of experiments. Conversely, groups that were exposed to 3,600 and 6,000 ppm toluene showed significant decrements in performance as compared to the control (air-exposed) group which interfered with our ability to detect changes in response inhibition. At the highest concentration, there was a breakdown of responding with animals responding at only 40%–45% of their baseline control levels. At the same point during the exposure period when the 3,600 ppm group began to develop behavior patterns characteristic of tolerance (i.e., increases in free rewards and FR resets), the 6,000 ppm group continued to decline in their ability to respond and obtain free milk rewards reaching a depressed baseline significantly below their training baseline. Even 22–23 hours after exposure, these mice were only pressing the lever at rates approximately half of that of their own control values collected prior to any toluene exposure. This decrease in lever-pressing indicates a persistent motor depression or impairment, or a possible lack of motivation following repeated toluene exposure. It is possible that this very high concentration of toluene interfered with learning and/or memory components which prevented the animals from remembering previously understood patterns of operant responding and preventing a valid test of response inhibition, or interpretations of “impulsivity.” Evidence from studies of psychological tests, as well as abusers’ testimonies, in fact report that chronic solvent inhalation results in apathy as well as various learning, memory and visuospatial deficits [26,29,39]. The current data support those findings.

While both higher dose groups showed an initial decrease in the number of free milk rewards, midway through the exposure period (at approximately 20 days) the two groups’ patterns of behavior diverged. The animals exposed to 6,000 ppm toluene continued to decline in their

ability to obtain free milk and in the number of FR Resets produced. In contrast, after the initial impairments in performance in both these measures, the 3,600 ppm-exposed animals dramatically increased the number of free rewards earned and the number of FR-Resets made, suggesting the development of tolerance to this concentration of toluene and a return to normal function. Such tolerance could be considered advantageous to the animal, consistent with theories emphasizing the adaptive value of tolerance [46]. With 20 consecutive days of exposure, animals would have had ample opportunity to adapt to the learning and/or motor impairments produced by previous toluene exposures. However, these two measures did not increase in the 6,000 ppm group, suggesting that the sedation or toxicity produced by this highest concentration of toluene may have been too great for these animals to effectively adapt. It is also possible that the duration of testing (30 min) was too brief and that tolerance may have developed if the animals had been given more time on the task.

While the present results are comparable to other studies demonstrating development of tolerance after repeated exposure to inhalants, it is important to note that tolerance development in the present investigation was observed ~22 hours after toluene exposure, at a time when toluene should have been completely cleared from both the body and brain. In contrast, many previous investigations have demonstrated that initial inhalant exposure impairs behavioral function when tested immediately after exposure and that tolerance is seen in a recovery of function. For example, after repeated exposures (two 3-day exposures) to 4500 ppm inhaled toluene, tolerance develops to impaired performance in a delayed matching-to-sample behavioral task [43]. Bushnell and Oshiro [9] reported that a moderate degree of tolerance develops to the effects of inhaled trichloroethylene in a signal detection task and Rees et al. [38] reported similar findings after repeated toluene exposures as measured by accuracy in signaled and unsignaled fixed consecutive number procedures. Bushnell and Oshiro [9] also found evidence for an adaptive learning component of tolerance to the effects of trichloroethylene with greater tolerance being observed when rats were exposed to trichloroethylene during rather than after performance of a signal detection task that resulted in reinforcement loss during exposure. Further work by this group [37] clearly implicates reinforcement loss as an important factor in the degree of tolerance development with trichloroethylene exposure. Additionally, Bowen and Balster [4] recently reported that tolerance was more likely to occur if the initial effects of 1,1,1-trichloroethane (TCE) produced disruptions in behavior, but when the effects of TCE were not disruptive (e.g., increased motor activity), sensitization occurred. However, other studies have shown no evidence for the development of tolerance to repeated inhalant exposure. For example, little or no evidence of tolerance was demonstrated for the effects of repeated toluene or TCE on response rates that result in loss of reinforcements [31,33]. This was true for FR schedules of reinforcement as well as for differential reinforcement of low rates of responding. Perhaps most relevant to the present findings, Moser and Balster [31] reported no evidence for tolerance in operant performance after 6,000 ppm toluene was administered before the session for 30 min daily, 7 days a week for 7 weeks, even though this pattern of toluene exposure reduced reinforcement rates. Clearly, the conditions under which behavioral tolerance to toluene (or any of the other abused solvents) can be observed needs further exploration.

While some indication for changes in response inhibition were demonstrated in mice *during* the binge patterns of exposure, there appeared to be a greater indication for this phenomenon *after* exposure during the recovery phase for the lowest concentration toluene group. This change in response inhibition would result in animals resetting the FR component more frequently, causing the animals to continue to work harder for fewer potential rewards and pass on higher rates of free rewards, while animals that inhibit their responses continue to receive free reinforcement, albeit at incrementally increasing delays. Evidence for this is primarily in the performance of the 1,000 ppm toluene group as compared to the control group on their differences in the numbers of FR resets (Figure 3, right panel), a variable which has been

interpreted to indicate impulsive behavior in rats [7]. It is notable that this type of deficit was not observed in the other toluene exposure groups, suggesting a biphasic dose response from toluene exposure. While this increase in FR resets was not observed for the other toluene groups, there was other evidence of changes in response efficiency. As illustrated in Figure 6, the 3,600 ppm toluene-exposed animals appeared to use less efficient response patterns throughout both the exposure and recovery periods which resulted in more responses for fewer reinforcers. One possible explanation for this is that these animals may have been unmotivated to complete the first FR15 which would have delayed entry into the wait component and any free reinforcement. As a result, these animals would have had to produce more responses in a shorter amount of time in an attempt to regain these reinforcements. This delay or lack of motivation to complete the first FR15 was even more evident in the highest dose of 6,000 ppm in which the animals were simply unable to perform. Additional support for a decrease in motivation comes from the decreases in weight gain that were observed for both the 3,600 ppm and 6,000 ppm toluene groups which parallels the decreases that were observed in operant performance.

Although the mechanisms through which toluene exerts its effects on the brain are only partially understood, the idea that this solvent may act as a stimulant is not novel. At concentrations similar to those encountered in abuse settings, inhaled toluene tends to produce a profile of effects that advance from motor excitation at low concentrations (i.e., 500–4,000 ppm) to sedation, motor impairment and anesthesia at higher concentrations of 6,000–15,000 ppm [3, 19,24,47]. The effects of toluene on operant behavior show a similar pattern. For example, Geller et al. [16] has shown that one-hour exposures to low levels of toluene (150 ppm) resulted in increased responding under a multiple FR-FI schedule of reinforcement. Similar increases in response rates have been described for toluene under a DRL component in rats trained to respond under a multiple FR-DRL schedule [10]. It is possible that the groups exposed to 1,000 ppm and 3,600 ppm experienced stimulatory effects from toluene exposure (with little evidence of tolerance to these stimulatory effects). Therefore, these data may lend support to the implication that long term, low level toluene exposure in humans may be similar to these and other drugs of abuse, with stimulatory properties [2].

In summary, inhalant abuse continues to be a worldwide public health concern. The effects of these toxic drugs of abuse, especially toluene, are not well understood and pose serious health risks to users. In the current study, daily, repeated, brief, high-concentration toluene exposures typical of abuse resulted in long-lasting motor deficits in mice at the higher concentrations (3,600 and 6,000 ppm) suggesting long-term brain damage, possibly resulting from cerebellar damage or cortical cell loss. Since these effects are common to other drugs of abuse such as alcohol, there is reason to be concerned about toluene's ability to act as a "gateway" drug leading to more serious and long-lasting drug abuse problems. Future research (e.g., trials-based learning/memory tasks) is needed to more thoroughly elucidate toluene's impact on cognition and behavior.

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Figure 1.
Diagram of “waiting for reward” paradigm (adapted from Brockel and Cory-Slechta, 1998).

Figure 2.

Effects of air and toluene exposure on the number of "free" rewards earned both during exposure (left panel) and during recovery (right panel). Each point represents the mean % of control ($\pm$ SEM) of rewards earned during a 30-min session collapsed in four day blocks. $N = 10$ mice for each group.

Figure 3.

Effects of air and toluene exposure on the number of FR resets during exposure (left panel) and during recovery (right panel). Each point represents the mean % of control ($\pm$ SEM) of FR resets during a 30-min session collapsed in four day blocks. N = 10 mice for each group.

Figure 4.

Effects of air and toluene exposure on the mean wait time both during exposure (left panel) and during recovery (right panel). Each point represents the mean % of control ($\pm$ SEM) of the average of the individual waiting times prior to each FR reset collapsed in four day blocks. $N = 10$ mice for each group.

Figure 5.

Effects of air and toluene exposure on response rates both during exposure (left panel) and during recovery (right panel). Each point represents the mean % of control ($\pm$ SEM) of the number of responses/second during a 30-min session collapsed in four day blocks. N = 10 mice for each group.

Figure 6.

Effects of air and toluene exposure on the number of responses per reward earned both during exposure (left panel) and during recovery (right panel). Each point represents the mean % of control ($\pm$ SEM) of the number of responses per reward earned during a 30-min session collapsed in four day blocks. $N = 10$ mice for each group.

TABLE 1

BASELINE CHARACTERISTICS (means ± sem)

Level of Toluene (ppm):	0	1,000	3,600	6,000	p
"Free" rewards	102.63 ± 6.04	118.35 ± 7.22	114.91 ± 5.41	114.88 ± 7.09	p=0.35
FR resets ^a	25.61 ± 4.06	28.54 ± 2.26	51.73 ± 6.53	30.67 ± 3.39	p<0.01
Mean Wait Time	54.32 ± 8.54	49.00 ± 5.99	96.66 ± 34.78	61.15 ± 12.39	p=0.30
Response Rate ^b	0.47 ± 0.02	0.32 ± 0.03	0.26 ± 0.01	0.32 ± 0.02	p<0.01
Responses per Reinforcer ^c	2.76 ± 0.09	2.39 ± 0.16	2.24 ± 0.24	3.34 ± 0.15	p<0.01

^a Mice assigned to the 3,600 ppm group had significantly more resets than any of the other groups.

^b Mice assigned to the 0 ppm group had significantly higher response-rates than any of the other groups.

^c Mice assigned to the 6,000 ppm group had a significantly higher response per reinforcer than mice assigned to the 1,000 and 3,600 ppm groups.