Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 3 (2014), Article ID 235841, 9 pages
doi:10.4303/jdar/235841

Research Article

Long-Term Behavioral Consequences of Prenatal Binge Toluene Exposure in Adolescent Rats*

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Received 11 December 2013; Revised 9 January 2014; Accepted 27 January 2014

Abstract The continued abuse of inhaled organic solvents, especially among women of childbearing age, raises the risk of long-term behavioral effects of maternal toluene abuse. In this study, the effects of short-term exposures to high toluene concentrations (i.e., "binges") were tested in independent groups of adolescent rats with different toluene treatments: (a) acute: 30-day-old animals exposed for 30 min to air (A) or 6,000 ppm toluene (T); (b) prenatal and postnatal: rats exposed to T or A from gestation days 8–20 and re-exposed to T or A from postnatal day (PN) 22 to PN30 (A/A, T/A, A/T, and T/T, resp.). On PN30, animals were evaluated in different tests. Postnatal toluene exposure produced anxiolytic-like effects in the burying behavior test, and the T/T group received the highest number of electrical shocks. Antinociception was observed in the T, A/T, and T/T groups in the hot-plate test. All toluene treatments impaired short-term memory in the object recognition test, but only postnatal exposure impaired long-term memory in the passive avoidance test. Sensitization occurred in the T/T group in locomotor activity. These results indicate that prenatal exposure to a concentration of toluene that does not produce evident malformations can modify behavioral toluene's effects in adolescent rats.

Keywords inhalants; solvents; toluene; prenatal exposure; adolescence; sensitization

1. Introduction

Abuse of volatile substances is a hazardous behavior that has been documented throughout the world [19,33,42]. Once observed almost entirely in males, volatile substance abuse has increased in females and the gender gap is narrowing [48]. Because volatile substance abuse has been documented to progress into adulthood, there are concerns that abuse by pregnant women may be rising [5,37,41] with potential adverse pregnancy outcomes, including developmental delays and neurobehavioral problems in children born to inhalant abusers [6,17,27,36].

Merit for these concerns comes from several reports that have shown that toluene abuse by pregnant women results in numerous developmental disorders and malformations in their children [1,2,25,28,29]. Women who abused toluene during their pregnancy gave birth to infants who were typically premature, stunted in their growth and had microcephaly. These infants also had distinct dysmorphology including deep-set eyes, small face, low-set ears, micrognathia, spatulate fingertips, and hypoplastic fingernails [2,25,28,29,32,54]. Also, exposure to very high levels of abused solvents like toluene during pregnancy has been reported to result in fetal or infant death [2,25,28,29,32,54,60].

Very little is known clinically or preclinically about the effects of prenatal toluene exposure. Only a handful of studies have examined gross dysmorphology following patterns of prenatal toluene exposure that mimic inhalant abuse (i.e., repeated, high dose short exposures) [8,10,12,26,59]. In previous work examining prenatal binge toluene exposure, repeated 15 min (8,000 or 12,000 ppm) exposure twice a day (30 min total per day) from Gestation Day 8 (GD8) through GD20 produced significant increases in total minor and gross malformations, including reduced body weight, soft tissue anomalies (e.g., missing eyes), and skeletal abnormalities (shortened and/or missing digits and missing limbs, reduced skeletal ossification), "runting" and fetal or postnatal death [8,51]. Growth deficits were more prevalent when repeated exposures to high concentrations were increased to 30 min binge exposures in rats [11] and mice [51]. This pattern of prenatal exposure to toluene also resulted in a higher incidence of gross morphological anomalies including

*This article is a part of a Special Issue on "Advances in the Neurobiological Basis of Inhalant Abuse." Preliminary versions of the papers featuring this special issue were originally presented at the 4th Meeting of the International Drug Abuse Research Society (IDARS) held in Mexico City, April 15–19, 2013.

shortened and/or missing digits and missing limbs [12]. In all of these studies, in utero toluene binge exposures resulted in significant increases in poor perinatal outcome (i.e., a combination of malformations, "runting," and neonatal death) without affecting maturational milestones.

While the above studies have focused on fetal development, little is known about the long-term effects of in utero toluene exposure on behavior, especially during adolescence. This is an important period because of the increased drug use in humans in this age group. During adolescence, numerous neural changes occur [50] including an increase in connectivity between the nucleus accumbens and the prefrontal cortex [20]. Widespread changes in the brain during adolescence have also been linked to greater long-term deleterious effects of drug use than use started at later ages [52,53]. To our knowledge, only one study has examined the long-term effects of in utero toluene exposure on adolescent behavior [14]. In this study, prenatal toluene exposure resulted in small alterations in spontaneous activity as compared to nonexposed rats with prenatal exposure to 12,000 ppm toluene resulting in significant hyposensitivity to the locomotor stimulatory effects of the amphetamine challenge in male but not female rats on PN28 [14]. These results suggest that prenatal exposure to high concentrations of toluene through inhalation can alter later behavior in rats. To our knowledge, there are no studies that have analyzed the combination of gestational exposure and postnatal chronic exposure to toluene in adolescent rats. Therefore, in the present study we analyzed the effects of short-term, high-concentration toluene exposure (6,000 ppm) in rats exposed prenatally and/or during adolescence on locomotor activity, anxiety-like response, nociception and learning. This concentration of toluene was chosen because abuse in humans typically involves > 20 deep inhalations of very high solvent concentrations (likely greater than 5,000 ppm) over a very short period of time (10–15 min) [16,40,61]. Also because 6,000 ppm toluene produces clear effects in several preclinical behavioral tests [9]. We hypothesized that prenatal toluene exposure would have an adverse impact in the behavior of adolescent rats re-exposed to toluene at a concentration that is not high enough to produce malformations.

2. Materials and methods

2.1. Animals

Wistar rats from our breeding facilities were used in this study. Animals were individually housed in polycarbonate cages with wood chip bedding, with ad libitum access to drinking water and food under controlled conditions of temperature ($22 \pm 2\,^{\circ}$C) and light (12:12 PM light:dark cycle). All experimental procedures were approved by our local Ethics Committee on Animal Experimentation (Protocol No. 380-07), and were in accordance to regulations established in the Mexican official norm for the use and care of laboratory animals "NOM-062- ZOO-1999" and ethical standards for investigation of experimental pain in animals [67].

Rats were allowed to acclimatize for one week before mating; vaginal smears were obtained daily to register the stage of the cycle. Adult rats (200–250 g) were placed overnight in harems of two females and one sexually experienced male. The presence of semen in vaginal smears confirmed pregnancy and when positive, it was considered gestational day zero (GD0).

2.2. Toluene exposure

Individual pregnant or adolescent rats were placed into a static exposure chamber to be exposed to air (control) or 6,000 ppm toluene (99.8% high-performance liquid chromatography grade; Sigma-Aldrich, Mexico) for 30 min, twice a day, between 07:00 AM and 16:00 PM. Exposures were conducted under a fume hood to ventilate toluene vapors away from laboratory personnel. Toluene concentrations and exposure times were chosen to mimic binge patterns of toluene exposure, which are typical of intentional solvent intoxication. The chambers consisted of sealed 29-L cylindrical glass jars covered with acrylic lids with injection ports. The lids were equipped with a fan and a stainless steel mesh box holding filter paper that projected into the jar. After placing a rat into the chamber, the lid was replaced and a calculated amount of solvent was added to the filter paper from which the fan volatilized the solvent. Calculated nominal concentrations were confirmed with a gas chromatograph coupled to a mass spectrophotometer detector (Model GCQ, Finnigan MAT, San Jose, CA, USA) and a PONA Chromatography column of 50 m length, 0.25 mm internal diameter, and 0.5 μm film (Hewlett Packard, Palo Alto, CA, USA). At the end of the exposure period, dams were returned to the vivarium into their home cages until the next exposure. Chambers were routinely cleaned between animal exposures.

2.3. Behavioral evaluation

The evaluation of anxiety-like behavior (burying behavior test), nociception (hot-plate test), long-term, short-term memory (step-through inhibitory avoidance task and object recognition test), and locomotor activity was conducted at PN30 in all groups, immediately after the last (or only) exposure to air or toluene. All the experimental sessions were videotaped and analyzed by an observer unaware of treatment conditions.

2.3.1. Burying behavior test

Rats were individually placed in a cage ($15 \times 24 \times 11$ cm) with an electrified prod (7-cm long) emerging from one of the walls 2 cm above the bedding material consisting of fine sawdust. Every time the animal touched the prod it received

an electric shock of 0.3 mA. The source of the shock was a constant current shocker (La Fayette Instruments Co., model 5806) and the prod remained electrified throughout the test. Immediately after introducing the animal in the cage, behavior was registered for 10 min. Once the animal received the first shock, it typically moved towards the prod recognizing it as the aversive stimulus. The animal would then spray and push a pile of bedding material ahead with rapid alternating movements of its forepaws. The parameters registered were the cumulative burying behavior (cumulative time, in seconds, that the animals spent burying the prod) and the total number of shocks received during the test. Both a decrease in the cumulative time of burying behavior and an increase in the number of shocks were interpreted as anxiolytic-like responses [56,57]. It is worth mentioning that false positives can occur in this test if animals are unable to feel the shock [55] or to recognize the prod as the source of the aversive stimulus due to learning impairment [43,67].

2.3.2. Hot-plate test

For nociception testing, each rat was introduced into a glass cylinder (20 cm diameter and 25 cm height) placed at the center of a metal plate adjusted at 53 ± 0.5 °C. Within several seconds, the animal typically displayed the forepaw lick behavior, which is a specific response evoked by the thermal stimulus. If this response did not occur, the test was concluded after 30 s to prevent tissue damage. Increases or decreases in the latency to forepaw lick indicated antinociception and pronociception, respectively [65].

2.3.3. Step-through inhibitory avoidance task

This test evaluated long-term, declarative memory and is based on the natural dark-seeking behavior of rats. It is a fear-motivated task in which the rat refrains from stepping through a door to an apparently safer dark compartment previously (-24 h) linked to a punishment [34,35]. The apparatus had two acrylic compartments ($9 \times 15 \times 32$ cm, each), one illuminated and one dark, separated by an automatic door. The illuminated side had a 60-watt light bulb. The floor of both compartments was made of stainless steel rods (3 mm diameter) spaced 1 cm apart and the floor of the dark chamber could be electrified. On habituation day, animals were placed in the lighted compartment for 30 s, the door was opened, and the latency to step-through was measured, after which the animal was returned to its home cage. The training session (acquisition trial) was conducted the next day. A single rat was placed in the illuminated compartment for 30 s, and thereafter the door was opened. Once the animal entered the dark compartment, the door was closed and an electric foot-shock (0.8 mA, 1 s in duration) was delivered through the grid floor. Ten seconds after receiving this shock, the animal was removed from the dark compartment and returned to its home cage. Twenty-four hours later in the retention trial, the rat was placed again in the illuminated side; 5 s later the door was opened and the step-through latency was measured. Animals that failed to enter the dark compartment within 5 min were removed from the apparatus and assigned a ceiling score of 300 s.

2.3.4. Object-recognition test

This test is based on the spontaneous behavior of rats to differentially explore familiar and new objects [21] and it is used to study short-term, declarative memory, and attention. One day prior to training, animals were habituated to the arena ($44 \times 34 \times 20$ cm) for 5 min in the absence of objects. During the training session, each rat was allowed to explore two identical objects (325-mL red cans or 250-mL clear plastic bottles) located in the back corners of the arena during 5 min. Exploration was defined as the animal reaching or sniffing the object at a maximal distance of 2 cm. Rats that did not explore the objects for at least 15 s (less than 10%) were dismissed to avoid false results due to poor basal exploratory activity. After training, each rat was returned to its home cage and 30 min later it was placed again in the arena for testing. During this retention trial, one of the objects was changed by a novel one, and the cumulative time spent by the animal exploring each object was recorded for 5 min. Results are expressed as the recognition index (RI), which results from dividing the novel object exploration time by the total exploration time. An RI value close to 0.5 indicates that animals spent a similar amount of time exploring the familiar and the novel object, while RI values greater than 0.5 suggest a preference to explore the novel object over the familiar one.

2.3.5. Locomotor activity test

Motor activity was evaluated in an arena divided into 12 equal quadrants of 11×11 cm each, by recording the number of quadrant crossings per minute during 5 min.

2.4. Experimental design

2.4.1. Acute exposure

Two groups were included as controls: adolescent animals (30 days of age) exposed only once for 30 min to air (A) or 6,000 ppm toluene (T) and tested immediately after exposure in each behavioral paradigm ($n = 10$ per group). Each animal was evaluated only once. To control for stress, rats were habituated for two consecutive days to the exposure chamber for 30 min with the fan turned on.

2.4.2. Prenatal exposure

Individual pregnant females were exposed to air (control group) or 6,000 ppm toluene from GD8 through GD20, twice daily (7:00 AM and 16 h, 30 min each). Rats were placed into their home cages in the vivarium with ad libitum access to food and water between exposures.

2.4.3. Postnatal exposure protocol

Beginning on GD20-21, dams were checked at 07:00 AM, 13:00 PM, and 19:00 PM to monitor the time of birth. For animals born between 19:00 PM and 8:00 AM, the time of birth was recorded at 24:00 PM. The day of birth was designated as postnatal day (PN) 0 and gestational length was calculated accordingly. Pups were weaned on PN21 and housed in groups of 5–6 same-sex littermates (or other animals from the same age and treatment) with ad libitum access to food and water. To avoid possible sex differences, only male pups were used for this study. They were individually re-exposed for 30 min to air or 6,000 ppm toluene, twice a day, 7 h apart, from postnatal day (PD) 22 to PD30.

With this design, four treatment groups were established: prenatal air + postnatal air (A/A); prenatal air + postnatal toluene (A/T); prenatal toluene + postnatal air (T/A); prenatal toluene + postnatal toluene (T/T).

2.5. Data analysis

Because data were not normally distributed, nonparametric statistical tests were used. Differences between treated and control acute groups (A vs. T) were analyzed using the Mann-Whitney U test. This test was also used to compare A versus A/A. A Kruskal-Wallis analysis of variance (ANOVA) followed by Dunn's test was utilized to compare all toluene treated groups. An additional Kruskal-Wallis ANOVA was performed among A/A, T/A, A/T, and T/T. Post hoc comparisons were done using Dunn's test. All statistical analyses were conducted using Sigma-Plot program (version 12, Jandel Scientific).

3. Results

Animals that were prenatally exposed to toluene ($n = 100$; 50 from T/A groups and 50 from T/T groups) were examined at birth to look for evident skeletal or soft tissue alterations. As expected, gestational exposure to 6,000 ppm toluene did not produce craniofacial or morphological alterations in the offspring.

3.1. Prenatal binge toluene exposure did not modify toluene's anxiolytic-like actions in the burying behavior test, but increased the number of electric shocks received by adolescent animals re-exposed to toluene

Figure 1(a) shows the effects of different schemes of toluene exposure (acute, prenatal and/or postnatal) in the time spent by adolescent rats burying the electrified probe. Acute toluene (T) exposure significantly decreased the burying behavior with respect to control rats exposed to air (A). Chronic exposure to toluene during adolescence, alone (A/T) or in combination with prenatal toluene treatment (T/T) also decreased the burying behavior. Prenatal toluene exposure per se (T/A) had no effect.

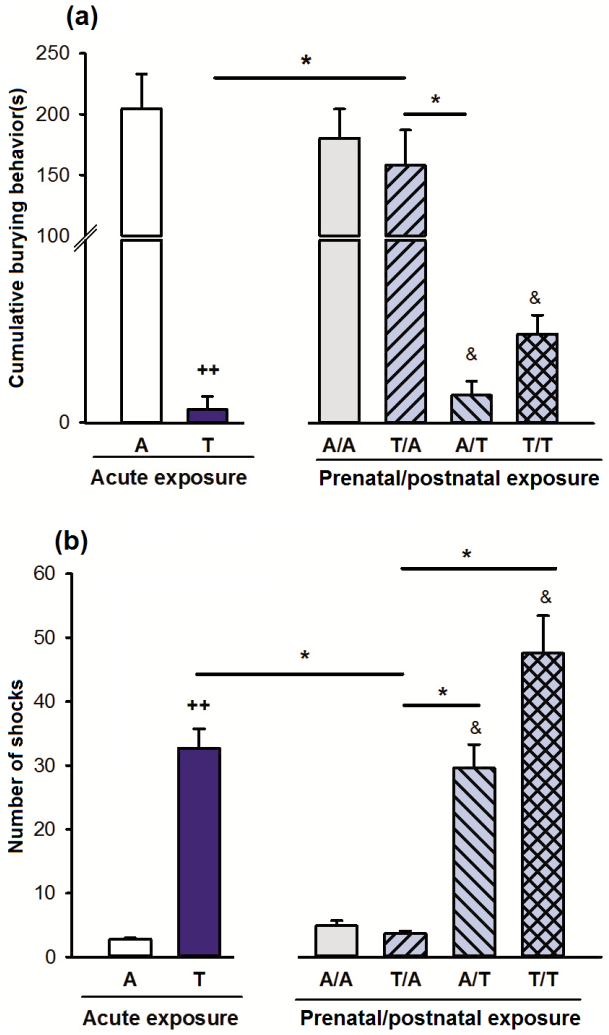

Figure 1: Acute and chronic exposure to toluene decreases cumulative burying behavior (a) and increases the number of shocks (b) received in the burying behavior test. A = air, T = acute toluene exposure (6,000 ppm, 30 min); $^{++}P < .01$ (Mann-Whitney U test). A/A, T/A, A/T, and T/T groups = the letters indicate prenatal/postnatal treatment (air or 6,000 ppm toluene); $^{\&}P < .05$ (comparisons vs. A/A, Dunn's test). $^{*}P < .05$ (comparison among toluene-treated groups, Dunn's test). Each bar represents the mean ±SEM of 10 rats.

As to the number of shocks (Figure 1(b)), acute toluene (T) produced a significant increase in this parameter. A similar effect was seen in rats chronically exposed to toluene during adolescence (A/T). Interestingly, the combination of prenatal and postnatal exposure (T/T) resulted in a significant increase in this effect with animals exposed in utero and re-exposed later as adolescents receiving an average of almost 50 shocks per session. This value was significantly higher than those observed in T (+31.23%) and A/T (+37.77%). Prenatal toluene exposure by itself had no effect.

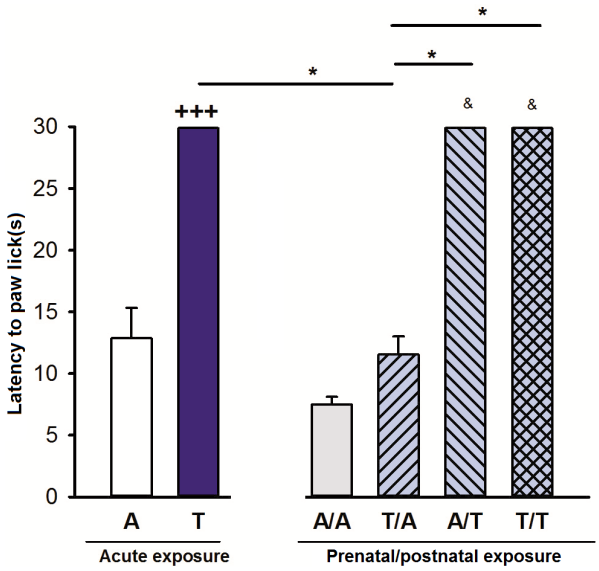

Figure 2: Acute and chronic toluene exposure increases nociception in the hot-plate test. A = air, T = acute toluene exposure (6,000 ppm, 30 min); $^{+++}P < .01$ (Mann-Whitney U test). A/A, T/A, A/T, and T/T groups = the letters indicate prenatal/postnatal treatment (air or 6,000 ppm toluene); $^{\&}P < .05$ (comparisons vs. A/A, Dunn's test). $^{*}P < .05$ (comparison among toluene-treated groups, Dunn's test). Each bar represents the mean ±SEM of 10 rats.

3.2. Toluene induced antinociception in adolescent rats and this parameter was not affected by prenatal treatment

Figure 2 shows the latency to forepaw lick behavior after exposure to different toluene treatments (acute, prenatal and/or postnatal) in adolescent rats in the hot-plate test. A significant increase in the latency to this response was observed after acute toluene (T) treatment with respect to control rats exposed to air (A). Similar results were seen in the groups chronically exposed or re-exposed to toluene during adolescence (A/T and T/T). No significant differences were observed among these three groups, as they all reached the cutoff latency value (30 s). Prenatal exposure to toluene (T/A) had no effect on the response to a thermal stimulus as the latency to paw lick was similar to that of control group.

3.3. Toluene impaired long-term memory in adolescent rats in the passive avoidance test, independently of prenatal toluene exposure

The effects of different schemes of toluene exposure in the passive avoidance task are depicted in Figure 3. Acute and chronic exposure to this solvent during adolescence, but not during gestation, impaired long-term memory in the passive avoidance test because animals entered the dark chamber in less than 20 s despite having received an electrical shock in that compartment 24 h before.

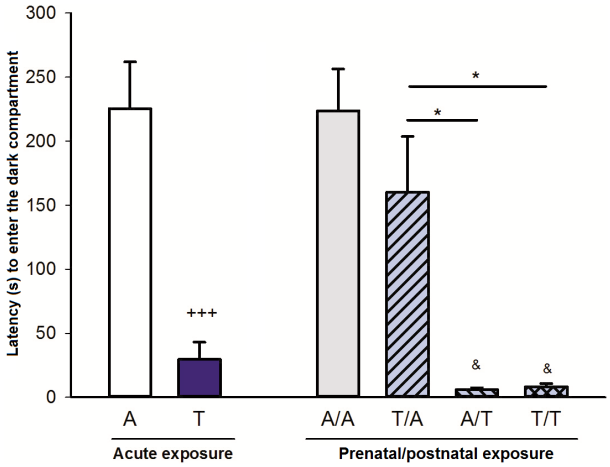

Figure 3: Acute and chronic toluene exposure impairs long-term memory in the passive avoidance test. A = air, T = acute toluene exposure (6,000 ppm); $^{+++}P < .001$ (Mann-Whitney U test). A/A, T/A, A/T, and T/T groups = the first letter indicates prenatal treatment (air or 6,000 ppm), and the second letter, postnatal treatment (air or 6,000 ppm toluene); $^{\&}P < .05$ (comparisons vs. A/A, Dunn's test). $^{*}P < .05$ (comparison among toluene-treated groups, Dunn's test). Each bar represents the mean ±SEM of 10 rats.

3.4. Prenatal binge toluene exposure impaired short-term memory in adolescent rats in the object recognition test

Figure 4 shows the effect of toluene on the object recognition index in adolescent rats. In this case, all toluene treatments, including prenatal exposure by itself (i.e., T, T/A, A/T, and T/T), impaired object recognition to a similar extent.

3.5. Prenatal exposure to toluene induced locomotor activity sensitization in rats re-exposed to toluene

Figure 5 shows the effects of different schemes of toluene treatment on locomotor activity in adolescent rats. As has already been described, acute toluene exposure resulted in a significant increase in locomotion with respect to control rats (group T). Prenatal toluene exposure (group T/A) did not affect general activity. Postnatal chronic exposure to toluene either alone or combined with prenatal exposure (groups A/T and T/T) significantly augmented locomotor activity. Chronic postnatal exposure by itself (A/T) approximately doubled the number of quadrant crossings with respect to acute toluene exposure (T). Interestingly, the combination of prenatal and juvenile exposures (T/T) further increased locomotor activity by an additional 50%.

4. Discussion

The present study evaluated the behavioral effects of toluene exposure in independent groups of adolescent rats (acute: 30-day-old animals exposed for 30 min to air (A)

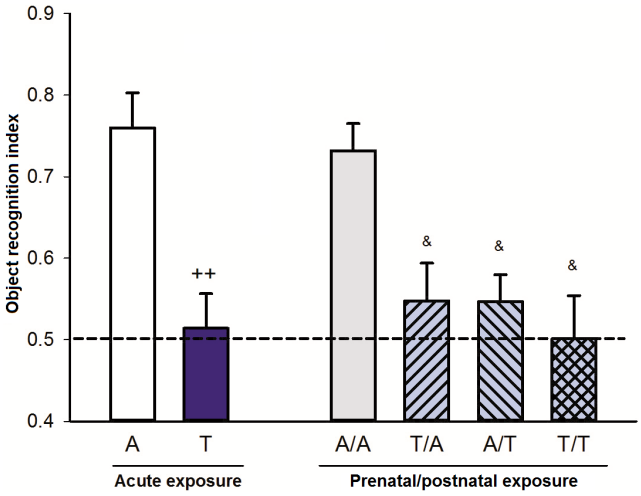

Figure 4: Acute, prenatal, and chronic toluene exposure impairs short-term memory in the object recognition test. A = air, T = acute toluene exposure (6,000 ppm); $^{++}P <$ .01 (Mann-Whitney U test). A/A, T/A, A/T, and T/T groups = the letters indicate prenatal/postnatal treatment (air or 6,000 ppm toluene); $^{\&}P < .05$ (comparisons vs. A/A, Dunn's test). Each bar represents the mean ±SEM of 10 rats.

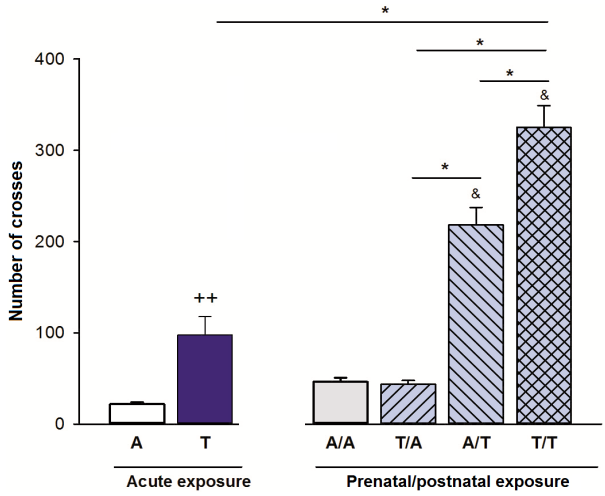

Figure 5: Acute and chronic exposure to toluene increases locomotor activity in the open field test. Prenatal toluene treatment produces sensitization to this effect. A = air, T = acute toluene exposure (6,000 ppm, 30 min); $^{++}P <$ .01 (Mann-Whitney U test). A/A, T/A, A/T, and T/T groups = the letters indicate prenatal/postnatal treatment (air or 6,000 ppm toluene); $^{\&}P < .05$ (comparisons vs. A/A, Dunn's test). $^{*}P < .05$ (comparisons among toluene-treated groups, Dunn's test). Each bar represents the mean ±SEM of 10 rats.

or 6,000 ppm toluene (T)) and compared these effects to prenatally and postnatally exposed offspring (rats exposed to T or A from gestation day 8–20 and re-exposed to T or A from postnatal day (PN) 22 to PN30 (A/A, T/A, A/T, and T/T, resp.)). Our results show that this binge pattern of prenatal toluene exposure, which was not high enough to produce evident physical malformations, was effective in significantly impairing short-term memory, modifying the response to an electrified prod in the burying behavior test, and sensitizing adolescent rats to the locomotor stimulatory effects of toluene.

4.1. Effects on memory

Mnemonic processes are particularly vulnerable to toluene effects. Learning and memory impairments are common among people chronically exposed to low solvent concentrations at work [18,66] or to high concentrations in order to experience psychoactive effects [22,66]. In preclinical studies, acute and chronic toluene exposure through inhalation or via IP results in impaired short-term memory in the object recognition test in mice [38,49,62] and rats [31]. Similar results have been reported in other experimental tests [58], which involve long-term memory or the use of relatively low toluene concentrations. Our results show that in utero exposure to toluene altered short-term memory in the object recognition test, but not long-term memory in a fear motivated test. This suggests that attention processes are more sensitive than emotional reactions to toluene's prenatal effects.

Deleterious effects on learning and memory have been associated with changes in the expression of NMDA receptor subunits NR1 and NR2 mRNA in the hippocampus of mice [18,62], inhibition of hippocampal neurogenesis [49], NMDA receptor antagonism, and nAChR antagonism [31]. It is necessary to evaluate whether these mechanisms are also involved in the impaired memory observed in rats exposed to toluene during gestation.

4.2. Effects on anxiety-like behavior

In the present study, acute and postnatal toluene exposure (T, A/T, and T/T groups) decreased the cumulative burying behavior, an effect which is generally considered as anxiolytic-like [57]. Similar antianxiety actions have been described in other animal models. For example, it has been reported that toluene reinstates lever responses that had been suppressed by punishment in adult rats [23,64] and reduces ultrasonic vocalizations in neonatal rats [3]. Also, in mice subjected to the plus maze test, toluene increases the total number of open arm entries and the total time spent on the open arms [15,39]. These effects are similar to those produced by classical anxiolytics [15] and potentiation occurs when toluene is combined with diazepam [23]. When comparing the effects caused by acute toluene exposure with chronic postnatal treatments, no significant differences were found; however, there was

a lower anxiolytic-like action in rats exposed to toluene during gestation and later re-exposed during adolescence (group T/T) compared with those that received toluene only once or several times during adolescence (T and A/T). This reduction could be indicative of tolerance development.

Another parameter registered in the burying behavior paradigm was the number of shocks that animals received during the 10 min test. An increase in this number is usually interpreted as a reduction in anxiety [57]; nevertheless, in a previous study we demonstrated that the unusual high number of shocks that animals received after acute toluene exposure was due, at least in part, to the animals' inability to recognize the electrified prod as the aversive stimulus [43]. When comparing the effect of different schemes of toluene administration on this parameter, we found that the combination of prenatal and postnatal toluene exposure (T/T) resulted in a significant higher number of shocks than what rats exposed acutely or chronically to toluene (T and A/T) received, indicating that sensitization occurred.

False positives can occur either when animals do not feel the electric shock in the burying behavior test [55] or fail to recognize the prod as the source of the aversive stimulus due to learning impairment [43]. Therefore, it is necessary to be cautious in the interpretation of these data and design experiments to determine if the observed sensitization is related to antianxiety like effects, impaired memory, decreased antinociception or a combination of these factors.

4.3. Locomotor effects

The locomotor stimulatory effects of toluene have been extensively evaluated through different approaches. In accordance with our results, other studies have shown that acute inhalation and systemic toluene administration increases locomotion [7,13,44]. The sensitization that we observed after postnatal chronic toluene exposure (A/T) is also in line with the observation that sensitization occurs after binge toluene exposure in mice and rats [4,13,14,30]. We did not see any effect with toluene prenatal exposure alone (T/A group), but this treatment sensitized animals to toluene's effects during adolescence (T/T group) to a degree that was higher than what we saw with postnatal toluene exposure (A/T).

Locomotor sensitization has been related to dopaminergic activation in the mesolimbic system [63]. In line with this idea, it has been demonstrated that toluene exposure increases dopamine release and dopaminergic neuron activity in the mesolimbic system [24,45]. The sensitization theory of addiction [46,47] suggests that an increase in locomotor activity can predict reinforcing drug effects and addiction liability. Based on these findings, it is reasonable to suppose that rats exposed in utero to toluene can have increased vulnerability to the reinforcing effects of toluene and addiction development.

It is interesting to note that in an earlier study, prenatal toluene exposure to 12,000 ppm toluene resulted in hyposensitivity to the locomotor stimulatory effects of an amphetamine challenge in male but not female rats on PN28 [14]; however, in that case rats were not re-exposed to toluene. This suggests that prenatal toluene exposure may have different impacts on early development of the monoaminergic systems that might become evident only under specific conditions (sex and postnatal treatment).

In conclusion, this study extends our animal model of prenatal exposure to abuse patterns of inhaled toluene by evaluating relatively brief, repeated exposures to high concentrations of toluene in adolescent rats via inhalation. We demonstrated that prenatally toluene-exposed rats without obvious physical malformations had impaired short-term memory in the object recognition test, received the highest number of electrical shocks in the burying behavior test and were sensitized to the stimulant locomotor effects of toluene during adolescence.

Acknowledgments The authors wish to thank Ms Isabel Beltrán Villalobos, Mr. Abraham Contreras, and Isaí Méndez for technical assistance. This work was partially supported by a grant from Conacyt-CB2010/155255 to CLR.

References

[1] H. Arai, M. Yamada, S. Miyake, S. Yamashita, H. Iwamoto, N. Aida, et al., *Two cases of toluene embryopathy with severe motor and intellectual disabilities syndrome*, No To Hattatsu, 29 (1997), 361–366.

[2] G. L. Arnold, R. S. Kirby, S. Langendoerfer, and L. Wilkins-Haug, *Toluene embryopathy: Clinical delineation and developmental follow-up*, Pediatrics, 93 (1994), 216–220.

[3] N. Bamat, S. Brunelli, M. Kron, A. Schulte, and B. Zimmerberg, *Behavioral effects of toluene in rats selectively bred for infantile vocalization rate*, Neurotoxicol Teratol, 27 (2005), 883–890.

[4] J. C. Batis, J. H. Hannigan, and S. E. Bowen, *Differential effects of inhaled toluene on locomotor activity in adolescent and adult rats*, Pharmacol Biochem Behav, 96 (2010), 438–448.

[5] F. Beauvais, J. C. Wayman, P. Jumper-Thurman, B. Plested, and H. Helm, *Inhalant abuse among American Indian, Mexican American, and non-Latino white adolescents*, Am J Drug Alcohol Abuse, 28 (2002), 171–187.

[6] S. E. Bowen, *Two serious and challenging medical complications associated with volatile substance misuse: Sudden sniffing death and fetal solvent syndrome*, Subst Use Misuse, 46 Suppl 1 (2011), 68–72.

[7] S. E. Bowen and R. L. Balster, *The effects of inhaled isoparaffins on locomotor activity and operant performance in mice*, Pharmacol Biochem Behav, 61 (1998), 271–280.

[8] S. E. Bowen, J. C. Batis, M. H. Mohammadi, and J. H. Hannigan, *Abuse pattern of gestational toluene exposure and early postnatal development in rats*, Neurotoxicol Teratol, 27 (2005), 105–116.

[9] S. E. Bowen, J. C. Batis, N. Páez-Martínez, and S. L. Cruz, *The last decade of solvent research in animal models of abuse: Mechanistic and behavioral studies*, Neurotoxicol Teratol, 28 (2006), 636–647.

[10] S. E. Bowen, J. D. Charlesworth, M. E. Tokarz, M. J. Wright Jr., and J. L. Wiley, *Decreased sensitivity in adolescent vs. adult rats to the locomotor activating effects of toluene*, Neurotoxicol Teratol, 29 (2007), 599–606.

[11] S. E. Bowen and J. H. Hannigan, *Binge toluene exposure in pregnancy and pre-weaning developmental consequences in rats*, Neurotoxicol Teratol, 38 (2013), 29–35.

[12] S. E. Bowen, S. Irtenkauf, J. H. Hannigan, and A. L. Stefanski, *Alterations in rat fetal morphology following abuse patterns of toluene exposure*, Reprod Toxicol, 27 (2009), 161–169.

[13] S. E. Bowen, S. Kimar, and S. Irtenkauf, *Comparison of toluene-induced locomotor activity in four mouse strains*, Pharmacol Biochem Behav, 95 (2010), 249–257.

[14] S. E. Bowen, M. H. Mohammadi, J. C. Batis, and J. H. Hannigan, *Gestational toluene exposure effects on spontaneous and amphetamine-induced locomotor behavior in rats*, Neurotoxicol Teratol, 29 (2007), 236–246.

[15] S. E. Bowen, J. L. Wiley, and R. L. Balster, *The effects of abused inhalants on mouse behavior in an elevated plus-maze*, Eur J Pharmacol, 312 (1996), 131–136.

[16] T. Brouette and R. Anton, *Clinical review of inhalants*, Am J Addict, 10 (2001), 79–94.

[17] J. A. Bukowski, *Review of the epidemiological evidence relating toluene to reproductive outcomes*, Regul Toxicol Pharmacol, 33 (2001), 147–156.

[18] S. Cairney, P. Maruff, C. Burns, and B. Currie, *The neurobehavioural consequences of petrol (gasoline) sniffing*, Neurosci Biobehav Rev, 26 (2002), 81–89.

[19] C. A. Dell, S. W. Gust, and S. MacLean, *Global issues in volatile substance misuse*, Subst Use Misuse, 46 Suppl 1 (2011), 1–7.

[20] T. L. Doremus-Fitzwater, E. I. Varlinskaya, and L. P. Spear, *Motivational systems in adolescence: Possible implications for age differences in substance abuse and other risk-taking behaviors*, Brain Cogn, 72 (2010), 114–123.

[21] A. Ennaceur and J. Delacour, *A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data*, Behav Brain Res, 31 (1988), 47–59.

[22] C. M. Filley, W. Halliday, and B. K. Kleinschmidt-DeMasters, *The effects of toluene on the central nervous system*, J Neuropathol Exp Neurol, 63 (2004), 1–12.

[23] I. Geller, R. J. Hartmann, V. Mendez, and E. M. Gause, *Toluene inhalation and anxiolytic activity: Possible synergism with diazepam*, Pharmacol Biochem Behav, 19 (1983), 899–903.

[24] M. R. Gerasimov, W. K. Schiffer, D. Marstellar, R. Ferrieri, D. Alexoff, and S. L. Dewey, *Toluene inhalation produces regionally specific changes in extracellular dopamine*, Drug Alcohol Depend, 65 (2002), 243–251.

[25] T. M. Goodwin, *Toluene abuse and renal tubular acidosis in pregnancy*, Obstet Gynecol, 71 (1988), 715–718.

[26] S. M. Gospe Jr., D. B. Saeed, S. S. Zhou, and F. J. Zeman, *The effects of high-dose toluene on embryonic development in the rat*, Pediatr Res, 36 (1994), 811–815.

[27] J. H. Hannigan and S. E. Bowen, *Reproductive toxicology and teratology of abused toluene*, Syst Biol Reprod Med, 56 (2010), 184–200.

[28] J. H. Hersh, *Toluene embryopathy: two new cases*, J Med Genet, 26 (1989), 333–337.

[29] J. H. Hersh, P. E. Podruch, G. Rogers, and B. Weisskopf, *Toluene embryopathy*, J Pediatr, 106 (1985), 922–927.

[30] D. J. Himnan, *Tolerance and reverse tolerance to toluene inhalation: Effects on open-field behavior*, Pharmacol Biochem Behav, 21 (1984), 625–631.

[31] A. Huerta-Rivas, C. López-Rubalcava, S. L. Sánchez-Serrano, M. Valdez-Tapia, M. Lamas, and S. L. Cruz, *Toluene impairs learning and memory, has antinociceptive effects, and modifies histone acetylation in the dentate gyrus of adolescent and adult rats*, Pharmacol Biochem Behav, 102 (2012), 48–57.

[32] A. G. Hunter, D. Thompson, and J. A. Evans, *Is there a fetal gasoline syndrome?*, Teratology, 20 (1979), 75–79.

[33] M. Hynes-Dowell, P. Mateu-Gelabert, H. M. Barros, and J. Delva, *Volatile substance misuse among high school students in South America*, Subst Use Misuse, 46 Suppl 1 (2011), 27–34.

[34] I. Izquierdo, J. H. Medina, M. R. Vianna, L. A. Izquierdo, and D. M. Barros, *Separate mechanisms for short- and long-term memory*, Behav Brain Res, 103 (1999), 1–11.

[35] M. E. Jarvik and R. Kopp, *An improved one-trial passive avoidance learning situation*, Psychol Rep, 21 (1967), 221–224.

[36] H. E. Jones and R. L. Balster, *Inhalant abuse in pregnancy*, Obstet Gynecol Clin North Am, 25 (1998), 153–167.

[37] T. L. Kurtzman, K. N. Otsuka, and R. A. Wahl, *Inhalant abuse by adolescents*, J Adolesc Health, 28 (2001), 170–180.

[38] B. F. Lin, M. C. Ou, S. S. Chung, C. Y. Pang, and H. H. Chen, *Adolescent toluene exposure produces enduring social and cognitive deficits in mice: An animal model of solvent-induced psychosis*, World J Biol Psychiatry, 11 (2010), 792–802.

[39] C. López-Rubalcava, R. Hen, and S. L. Cruz, *Anxiolytic-like actions of toluene in the burying behavior and plus-maze tests: differences in sensitivity between 5-HT$_{1B}$ knockout and wild-type mice*, Behav Brain Res, 115 (2000), 85–94.

[40] R. Marjot and A. A. McLeod, *Chronic non-neurological toxicity from volatile substance abuse*, Hum Toxicol, 8 (1989), 301–306.

[41] E. L. McGarvey, G. J. Clavet, W. Mason, and D. Waite, *Adolescent inhalant abuse: Environments of use*, Am J Drug Alcohol Abuse, 25 (1999), 731–741.

[42] M. E. Medina-Mora and T. Real, *Epidemiology of inhalant use*, Curr Opin Psychiatry, 21 (2008), 247–251.

[43] N. Páez-Martínez, S. L. Cruz, and C. López-Rubalcava, *Comparative study of the effects of toluene, benzene, 1,1,1-trichloroethane, diethyl ether, and flurothyl on anxiety and nociception in mice*, Toxicol Appl Pharmacol,, 193 (2003), 9–16.

[44] A. C. Riegel, S. F. Ali, and E. D. French, *Toluene-induced locomotor activity is blocked by 6-hydroxydopamine lesions of the nucleus accumbens and the mGluR2/3 agonist LY379268*, Neuropsychopharmacology, 28 (2003), 1440–1447.

[45] A. C. Riegel, A. Zapata, T. S. Shippenberg, and E. D. French, *The abused inhalant toluene increases dopamine release in the nucleus accumbens by directly stimulating ventral tegmental area neurons*, Neuropsychopharmacology, 32 (2007), 1558–1569.

[46] T. E. Robinson and K. C. Berridge, *The neural basis of drug craving: An incentive-sensitization theory of addiction*, Brain Res Brain Res Rev, 18 (1993), 247–291.

[47] T. E. Robinson and K. C. Berridge, *Addiction*, Annu Rev Psychol, 54 (2003), 25–53.

[48] SAMHSA, *Patterns and trends in inhalant use by adolescent males and females: 2002–2005*, the nsduh report, Substance Abuse and Mental Health Services Administration, U.S. Department of Health and Human Services, Rockville, MD, 2007.

[49] H. S. Seo, M. Yang, M. S. Song, J. S. Kim, S. H. Kim, J. C. Kim, et al., *Toluene inhibits hippocampal neurogenesis in adult mice.*, Pharmacol Biochem Behav, 94 (2010), 588–594.

[50] C. Sisk and J. Zehr, *Pubertal hormones organize the adolescent brain and behavior*, Front Neuroendocrinol, 26 (2005), 163–174.

[51] P. Soberanes-Chávez, C. López-Rubalcava, P. de Gortari, and S. L. Cruz, *Exposure to toluene and stress during pregnancy impairs pups' growth and dams' lactation*, Neurotoxicol Teratol, 40 (2013), 9–16.

[52] L. P. Spear, *The adolescent brain and age-related behavioral manifestations*, Neurosci Biobehav Rev, 24 (2000), 417–463.

[53] L. P. Spear, *Modeling adolescent development and alcohol use in animals*, Alcohol Res Health, 24 (2000), 115–123.

[54] C. Toutant and S. Lippmann, *Fetal solvents syndrome*, Lancet, 1 (1979), 1356.

[55] D. Treit, *The inhibitory effect of diazepam on defensive burying: Anxiolytic vs. analgesic effects*, Pharmacol Biochem Behav, 22 (1985), 47–52.

[56] D. Treit, *A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics*, Pharmacol Biochem Behav, 36 (1990), 203–205.
[57] D. Treit, J. P. Pinel, and H. C. Fibiger, *Conditioned defensive burying: A new paradigm for the study of anxiolytic agents*, Pharmacol Biochem Behav, 15 (1981), 619–626.
[58] M. von Euler, T. M. Pham, M. Hillefors, B. Bjelke, B. Henriksson, and G. von Euler, *Inhalation of low concentrations of toluene induces persistent effects on a learning retention task, beam-walk performance, and cerebrocortical size in the rat*, Exp Neurol, 163 (2000), 1–8.
[59] R. Warner, H. E. Ritchie, P. Woodman, D. Oakes, and M. Pourghasem, *The effect of prenatal exposure to a repeat high dose of toluene in the fetal rat*, Reprod Toxicol, 26 (2008), 267–272.
[60] L. Wilkins-Haug and P. A. Gabow, *Toluene abuse during pregnancy: Obstetric complications and perinatal outcomes*, Obstet Gynecol, 77 (1991), 504–509.
[61] J. F. Williams, M. Storck, American Academy of Pediatrics Committee on Substance Abuse, and American Academy of Pediatrics Committee on Native American Child Health, *Inhalant abuse*, Pediatrics, 119 (2007), 1009–1017.
[62] T. T. Win-Shwe and H. Fujimaki, *Acute administration of toluene affects memory retention in novel object recognition test and memory function-related gene expression in mice*, J Appl Toxicol, 32 (2012), 300–304.
[63] R. A. Wise and M. A. Bozarth, *A psychomotor stimulant theory of addiction*, Psychol Rev, 94 (1987), 469–492.
[64] R. W. Wood, J. B. Coleman, R. Schuler, and C. Cox, *Anticonvulsant and antipunishment effects of toluene*, J Pharmacol Exp Ther, 230 (1984), 407–412.
[65] T. L. Yaksh, *Pharmacology and mechanisms of opioid analgesic activity*, Acta Anaesthesiol Scand, 41 (1997), 94–111.
[66] M. Yücel, M. Takagi, M. Walterfang, and D. I. Lubman, *Toluene misuse and long-term harms: A systematic review of the neuropsychological and neuroimaging literature*, Neurosci Biobehav Rev, 32 (2008), 910–926.
[67] M. Zimmermann, *Ethical guidelines for investigations of experimental pain in conscious animals*, Pain, 16 (1983), 109–110.