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Gestational Toluene Exposure Effects on Spontaneous and Amphetamine-Induced Locomotor Behavior in Rats

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Abstract

The abuse of volatile organic solvents (inhalants) continues to be a major health concern throughout the world. Toluene, which is found in many products such as glues and household cleaners, is among the most commonly abused organic solvents. The neurobehavioral teratogenic sequelae of solvent abuse (i.e., repeated, brief inhalation exposures to very high concentrations of solvents) have not been examined thoroughly. In a preclinical model of inhalant abuse, timed-pregnant Sprague-Dawley rats were exposed to 0, 8,000, or 12,000 parts per million (ppm) for 15 min twice daily from gestation day 8 (GD8) through GD20. In the first experiment, separate groups of offspring were observed individually in an open-field on postnatal day 22 (PN22), PN42 or PN63. In the second experiment, other offspring given identical prenatal toluene exposures were observed in an “open-field” following an acute i.p. injection of amphetamine (0, 0.56, 1.78 mg/kg) on PN28. Automated measurements of distance traveled and ambulatory time were recorded. Prenatal toluene exposure resulted in small alterations in spontaneous activity compared to non-exposed rats. Prenatal exposure to 12,000 ppm toluene resulted in significant hyposensitivity to the locomotor stimulatory effects of the amphetamine challenge in male but not female rats on PN28. The results demonstrate that prenatal exposure to abuse patterns of high concentrations of toluene through inhalation can alter spontaneous and amphetamine-induced locomotor behavior in rats. The expression of these effects also appears to depend upon the postnatal age of testing. These results imply that abuse of organic solvents during pregnancy in humans may also produce long-lasting effects on biobehavioral development.

1. Introduction

Inhalant abuse is currently a leading form of drug abuse in the United States [26,44]. The most recent Household Survey estimates that 9.7% of Americans 12 years old and older deliberately

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abused inhalants at least once in their lifetimes and 0.9% did so in the previous year. For people between 18 and 25 years old, prime childbearing years, there was a 243% increase in the rate of first-time use between 1990 and 2001 [44]. It was previously estimated that more than half of all inhalant abusers are women of prime child-bearing age [41]. While the majority of users have come from lower socioeconomic status (SES) groups [10,31,38], where there is also a relatively higher risk for adolescent pregnancy [14], rates of inhalant abuse are increasing in other SES groups as well [52]. Because rates of inhalant abuse among young women in their childbearing years are so high [6,31,35], there are rising concerns about the potential negative impact of intentionally inhaled organic solvents on the unborn [10,29], as well as an increasing demand for research to improve our understanding of the teratogenic sequelae of organic solvent abuse during pregnancy.

Among the most commonly abused inhalants is the aromatic hydrocarbon toluene (methylbenzene; CAS 108-88-3). Toluene is relatively inexpensive and easily acquired, and is found in high concentrations in spray paints, glues, gasoline, and other household and industrial products. Gestational exposure to toluene is of particular concern because even relatively constant, low-level occupational toluene exposure can result in negative pre- and post-natal outcomes in offspring [for review, see 53]. In studies modeling *in utero* exposures to occupational levels of toluene (i.e., typically under ~1,500 ppm for 6 hrs/day), there are often small or inconsistent effects on gestational outcome, fetal growth and birth weight, postnatal maturation, and behavioral outcomes including locomotor activity, as well as effects on learning [e.g., 20, 23, 24, 28, 46]. It is reasonable to predict that the impact of abuse patterns of prenatal toluene exposure might be greater than those following occupational exposure patterns.

The rationale for these experiments is the continuing increase among pregnant women to inhale substances that often contain toluene [11,29]. Clinical cases of toluene-related embryopathy and malformations have been reported after toluene abuse by pregnant women (see review by Bowen and Hannigan, 2006 [9]). Very high levels of maternal solvent exposure typical of abuse can lead to perinatal death [1,53] and there are reports that surviving neonates show evidence of morphological teratogenicity [e.g., 29, 36]. At birth, affected infants were typically premature and/or growth restricted and microcephalic with severe facial dysmorphology (e.g., deep-set eyes, small face, low set ears, micrognathia), and spatulate fingertips and small fingernails [1,19,21,22,25,47]. The term “fetal solvent syndrome” has been adopted to describe this constellation of morphological and behavioral effects, following the model of fetal alcohol spectrum disorders (FASD) and comparing the phenotype of toluene embryopathy to the effects of prenatal alcohol exposure [29]. While the risk for fetal demise or major malformations appears to lessen as the concentration of toluene exposure lessens, a substantial risk for neurobehavioral sequelae may still remain [1,21]. Few studies have investigated the neurobehavioral effects of a clinically relevant rat model of binge patterned, gestational toluene abuse.

Using a preclinical model, we have shown that prenatal exposure to brief, abuse-level concentrations of toluene results in abnormal postnatal development and an increased likelihood of teratogenic outcomes [8]. Specifically, offspring of female rats exposed repeatedly to 15 mins of 8,000 ppm or 12,000 ppm of toluene twice per day during gestation display delayed postnatal development up to postnatal day 16 (PN16), decreased muscle tone to PN12, deficits in motor coordination assessed as negative geotaxis, and increased rates of physical abnormalities and “runting” [8]. To the best of our knowledge, the long-term effects of gestational exposure to high doses of toluene on post-weaning behavior have not been examined. The present two experiments assessed the impact of brief, repeated high-concentration toluene exposures during gestation on the development of spontaneous and

amphetamine-induced locomotor activity in rats. Offspring were assessed as juveniles (PN22 or PN28), in “adolescence” (PN42), and as young adults (PN63). The results with the present model show that gestational exposure to abuse levels of toluene may result in some long-term behavioral alterations in offspring.

2. Materials and Methods

2.1 Animals

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). Timed-pregnant female Sprague-Dawley rats were acquired on gestation day 4 (GD4; sperm plug positive = GD0) from Charles Rivers Breeding Laboratories (Portage, MI, USA) and across both experiments weighed a mean of 227 g (± 1.38 s.e.m.) on arrival. Pregnant rats were housed individually in polypropylene cages (52 cm $\times$ 28 cm $\times$ 22 cm) with hardwood chip bedding and had *ad lib* access to Rodent Lab Diet 5001 (PMI, Nutrition International, Inc., Brentwood, MO) and water when in their home cages. The only time the dams had no food or water was when they were in the lab for the toluene exposures. The AAALAC-approved vivarium was kept on a 12-h light/12-h dark cycle (lights on 0600 h).

The dams were weighed every other day beginning on GD4 until GD20. Toluene exposures occurred from GD8 – GD20 during the dams’ light cycle. Beginning on GD20, dams were inspected frequently between 0800 hrs and 2000 hrs for births with the day of birth designated as postnatal day 0 (PN0). The maximum reliable resolution for assessing gestation length was one half day. On PN1, the pups were weighed and examined for obvious physical abnormalities. Experimenters were kept blind to exposure condition during culling. Pups with any noticeable abnormalities or deemed “runts” were weighed and culled. All litters were then culled pseudo-randomly to 10 from the remaining pups, keeping 5 males and 5 females when possible. Offspring were housed with their dams until weaning on PN21 when all offspring were uniquely identified by ear punch. Dams were then sacrificed and the offspring re-housed in groups of 2 or 3 same-sex littermates or other rats from the same prenatal toluene concentration. All animals were unhandled and remained naïve to the testing procedures until their respective day of testing at PN22, PN42 or PN63, for Experiment 1, or PN28 for Experiment 2.

2.2 Inhalation exposure procedures

Dams were assigned to one of three gestational treatment groups, counter-balanced for maternal body weight on GD4. Exposure procedures were identical to those previously described [8]. Briefly, from GD8 to GD20, dams were weighed every other day and exposed daily via inhalation to 8,000 ppm or 12,000 ppm of toluene. An “air-only” control group received the same placement into the chamber without toluene (0 ppm). Dams were administered two 15-min inhalation exposures per day with a 2-hour interval between exposures. Vapor exposures were given in one of 4 sealed 36-liter cylindrical glass jars with Plexiglas lids and contained within a fume hood. During toluene exposure, dams were placed one at a time into the chambers onto a grid floor 20 cm from the bottom and 30 cm from the vapor diffuser in the lid. Chambers were sealed and toluene (0.0 mL for air controls, 1.36 mL for 8,000 ppm, 2.05 mL for 12,000 ppm) was injected through a solvent injection port onto a filter paper held in the stainless steel mesh diffuser. The injection port was sealed and a fan mounted directly above the filter paper volatilized and diffused the toluene. Following a 15-min exposure, chambers were evacuated before continuing with the next group of dams. Within a given cohort, the air-only (0 ppm) exposures were restricted to one chamber, but that chamber

was counterbalanced across cohorts. Single wavelength-monitoring infrared spectroscopy (Miran 1-A, Foxboro, Analytical, North Haven, CT) was used periodically to ensure consistent ambient toluene concentrations and proper chamber clearance.

2.3 Locomotor activity testing

Open-field locomotor activity was assessed for Experiment 1 on PN22, PN42, or PN63 in at most one male and one female from each litter at each age. Animals were weighed and transported from the vivarium to an isolated testing room and placed immediately into automated activity monitors (Opto-Varimex-3, Columbus Instruments, Columbus, OH) for one hour. The monitors were 70 cm × 70 cm × 30 cm “open-fields” with clear Plexiglas walls and equipped with 3 15-by-15 infrared photobeam arrays. Separate animals assessed for amphetamine-induced activity in Experiment 2 were transported similarly to the testing room on PN28 where they were injected i.p. with either D-amphetamine (0.56 mg/kg or 1.78 mg/kg; volume = 1 ml/kg) or vehicle (0.9% saline solution) and then placed immediately into the same activity monitors for two hours. Immediately following each session, animals were removed from the chambers that were then cleaned of debris and disinfected using a mild deodorant cleanser.

Testing sessions occurred between 1000 h and 1600 h in an isolated, test room dimly lit with a single red light and an ambient temperature of approximately 24°C. No experimenters were present during testing and continuous background white noise was provided to reduce environmental disruptions during testing and while preparing the animals for testing.

2.4. Statistical analysis

2.4.1. Maternal and pup variables—Maternal body weight was analyzed using a 3 × 8 two-way, repeated measures ANOVA with prenatal Treatment (0 ppm, 8,000 ppm & 12,000 ppm) and Gestation Day (GD4, GD8, GD10, GD12, GD14, GD16, GD18 & GD20) as factors. Weight gain, litter size, gestation length, pup weight and litter sex-ratio on PN1 were analyzed by one-factor ANOVAs with prenatal Treatment as the factor. Offspring development variables, including pup weight, were analyzed using litter as the unit of measure. The means of all male and female pups, respectively, within a litter were calculated for each outcome and used in the analyses. An alpha level of $p < 0.05$ determined statistical significance. Tukey's *B post hoc* contrasts were used as appropriate.

2.4.2. Activity variables—A maximum of one male and one female from each litter was used at each age in Experiment 1, or at each D-amphetamine Dose in Experiment 2, which was analyzed similarly. Spontaneous locomotor activity in Experiment 1 was quantified for 60 min, while amphetamine-induced locomotor activity in Experiment 2 was assessed for 120 min, both using the Auto-Track System Version 3.0A (Columbus Instruments, Columbus, OH). This system quantified total beam breaks in both the vertical and horizontal planes, specifically encoding into measures of distance traveled (cm; calculated from number of breaks of adjacent beams), ambulatory time (secs), and number of rears. Due to system constraints, the total number of rears was not assessed for PN22 animals. The automated various measures of activity were transformed into 5-min blocks over the duration of the session for analyses.

Activity measures for Experiment 1 were analyzed separately for each measure using 3 × 2 × 3 × 12 four-way, repeated measures ANOVAs with prenatal Treatment (0 ppm, 8,000 ppm & 12,000 ppm), Sex and Age (PN22, PN42 or PN63) as between-subjects factors, and 5-min Time block as the within-subjects factor. Activity measures for Experiment 2 were also analyzed separately for each measure using 3 × 2 × 3 × 12 four-way, repeated measures ANOVAs with prenatal Treatment (0 ppm, 8,000 ppm & 12,000 ppm), Sex and amphetamine

Dose (0, 0.56 or 1.78) as between-subjects factors, and 5-min Time block as the within-subjects factor. An alpha level of $p < 0.05$ determined statistical significance. Tukey's *B post hoc* contrasts were used as appropriate.

2.5. Chemicals

Toluene was purchased from Fisher Scientific (T-324, Fisher Scientific Co., Fairlawn, NJ), while the D-Amphetamine sulfate was purchased from Sigma-Aldrich and administered i.p. (0, 0.56, or 1.78 mg/kg) in a volume of 1 ml/kg. Saline (0.9%) solution was the vehicle.

3.0 Results

3.1 Maternal and litter characteristics

In response to exposure to either concentration of toluene, the dams showed initial qualitative increases in activity in the chambers (locomotion and rearing) followed by concentration-dependent decreases in activity. As the number of toluene exposures increased, the dams appeared to develop a tolerance to the activating effects of toluene, in that they remained ambulatory longer than they had during earlier exposures, and they recovered more quickly following removal from the exposure chambers. Following the first day of exposures to 12,000 ppm toluene, the dams were essentially immobile and appeared sedated, with post-exposure recovery taking ~20 minutes. By the final day, all dams showed rapid recovery in returning to pre-exposure levels of activity within ~10 minutes of removal from the chamber.

Experiment 1—Maternal and litter data for the 99 dams included in the three prenatal Treatment groups are presented in the top panel of Table 1. Maternal weight on GD4 was not significantly different across groups ($p > 0.05$). There was, of course, significant maternal weight gain during gestation in all groups, averaging 106.45 g, or a 48% increase over the initial weight on GD4, the day of arrival in the lab ($F(1,96) = 3037.14$, $p < 0.001$). There was a significant prenatal toluene Treatment by gestation Day interaction ($F(2,96) = 6.33$, $p < 0.003$). *Post hoc* analyses revealed that dams in both toluene-exposed groups gained significantly less weight than the air-exposed control dams. The toluene-exposed groups were not significantly different from each other ($p > 0.05$). There was also a significant main effect of prenatal Treatment on total litter size ($F(2,96) = 4.37$, $p < 0.01$), with the 8,000-ppm dams giving birth to significantly fewer pups than the 12,000-ppm dams.

Experiment 2—Maternal and litter data for the 87 dams included in the three prenatal Treatment groups are presented in the bottom panel of Table 1. Maternal weight on GD4 was not significantly different across groups ($p > 0.05$). As in Experiment 1, there was significant maternal weight gain during gestation in all groups, averaging 104.51 g, or 46% of initial body weight on GD4, the day of arrival in the lab ($F(1,84) = 1998.45$, $p < 0.001$). There was a significant prenatal toluene Treatment by gestation Day interaction ($F(2,84) = 3.54$, $p < 0.03$). *Post hoc* analyses revealed that both toluene-exposed groups gained significantly less weight than the air-exposed control dams ($p < 0.05$). The two toluene-exposed groups were not significantly different from each other ($p > 0.05$). No significant prenatal Treatment group differences were found in total litter size, sex ratio or mean gestation length.

3.2 Pup Body Weights

A total of 503 rat offspring were used for the activity experiments. Significant main effects were found for prenatal Treatment on PN1 body weight for Experiment 1 ($F(2,95) = 3.14$, $p < 0.05$; Table 1). *Post hoc* analysis revealed that the 12,000-ppm pups weighed significantly less than pups in the 0-ppm and 8,000-ppm groups on PN1. A significant main effect was also found for Sex, ($F(1,184) = 6.24$, $p < 0.05$), with female pups weighing less than their male

counterparts. There was a large overall main effect of Age as all offspring gained significant weight by weaning on PN21 ($F(1,93) = 1,422.17, p < 0.001$). Body weights of the toluene-exposed pups caught up to their air-exposed counterparts by PN21 when body weights were no longer significantly different among prenatal Treatment groups (0 ppm = 51.15 ± 0.82 ; 8,000 ppm = 45.81 ± 2.75 ; 12,000 ppm = 49.55 ± 1.78). The impact of prenatal toluene on body weight after PN1 may be underestimated because the “runts” were culled on PN1 and not included in subsequent analyses of body weight [cf, 8]. There was also a significant main effect of prenatal Treatment on PN1 body weight for offspring born to dams in Experiment 2 ($F(2,86) = 2.98, p = 0.05$; see Table 1), where the 12,000-ppm toluene group weighed ~4% less than the 0-ppm controls (Table 1). A significant main effect was also found for Sex, ($F(1,173) = 16.32, p < 0.01$), with female pups again weighing less than their male counterparts.

3.3 Locomotor activity

3.3.1.1. Distance Traveled There were no significant main effects or interactions involving prenatal toluene Treatment (p 's > 0.97 ; Figure 1). Significant main effects were observed for Sex ($F(1,192) = 16.75, p < 0.001$) and Age ($F(2,192) = 76.22, p < 0.001$), with females and older animals traveling further than males or younger animals, respectively (Figure 1, top panels). A significant Age $\times$ Sex interaction ($F(2,192) = 5.14, p < 0.001$) indicated that females traveled greater distances than males as they got older (data not shown).

3.3.1.2. Ambulatory Time The pattern of results for time spent moving generally paralleled those for distance traveled (see Figure 1, bottom panels). There were no significant main effects involving prenatal toluene Treatment (p 's > 0.51). A significant main effect was observed for Age ($F(2,192) = 98.39, p < 0.001$) with older animals being more active than younger animals. The Age $\times$ prenatal Treatment interaction approached significance ($p = 0.09$) due largely to the tendency for animals from the two toluene-exposed to be more active than air-exposed controls on PN22 ($p < 0.03$). In a further exploration of this marginal Age $\times$ prenatal toluene Treatment interaction, we conducted an exploratory follow-up analysis by adding to the design the one-hour ambulatory time data from the PN28 groups from Experiment 2 (below), animals that had only received saline injections and found that with the added precision on the Age factor, the Age $\times$ prenatal Treatment interaction reached significance ($F(6,269) = 2.10, p = 0.05$). *Post hoc* analyses indicated that this was due to the toluene-exposed animals being more active at PN22 as compared to air-exposed animals, whereas the toluene-exposed animals tested had either the same level or less activity than the air-exposed animals at all other ages.

3.3.1.3. Rearing As noted, rearing was not quantified at PN22. A significant main effect was observed for toluene Treatment ($F(2,140) = 3.01, p = 0.05$) with rats exposed prenatally to 8,000 ppm toluene having significantly fewer rears than both air-exposed controls and animals exposed prenatally to 12,000 ppm toluene (Figure 2). A significant main effect for Age ($F(1,140) = 7.75, p < 0.01$) was due to the PN63 animals displaying more rears than PN42 animals. A Sex $\times$ Time interaction was also significant, ($F(22,2112) = 2.37, p < 0.01$), which *post hoc* analysis showed was due to male animals greatly reducing their rearing activity after only ~25 min into the session, whereas female animals continued their rearing until ~45 min or so before decreasing movement to baseline (data not shown).

3.3.2.1. Distance Traveled A significant main effect was observed for amphetamine Dose ($F(2,229) = 118.47, p < 0.001$) at PN28. While there were no significant main effects or interactions involving prenatal toluene Treatment (p 's > 0.10), Figure 3 gives the appearance that the 12,000-ppm exposed males show a different amphetamine dose response than the other groups, however, the interaction did not reach significance ($p = 0.07$). No other main effects were significant (p 's > 0.26). A significant Sex $\times$ Time interaction ($F(23,5267) = 1.57, p < 0.05$)

and *post hoc* analyses indicated that female rats remained more active than males from ~45 min to the end of the session, collapsed across amphetamine Dose.

3.3.2.2. Ambulatory Time The basic pattern of results for time moving paralleled those for distance traveled although there were significant effects and interactions involving prenatal toluene exposure. The expected significant main effect was observed for amphetamine Dose ($F(2,229) = 195.44, p < 0.001$) with animals receiving amphetamine being more active than saline-treated groups (see Figure 4). A significant main effect was observed for prenatal Treatment ($F(2,229) = 2.99, p < 0.05$) with animals exposed prenatally to toluene (8,000 ppm) being more active than controls overall. A significant amphetamine Dose $\times$ prenatal Toluene interaction was also observed, ($F(4,229) = 2.36, p < 0.05$) which *post hoc* analyses indicated was due to toluene-pretreated animals (8,000 ppm) being more sensitive to the locomotor-stimulatory effects of the highest dose of amphetamine than other groups. As for distance traveled, males that had been exposed to 12,000-ppm toluene also appeared to be less sensitive to the locomotor-activating effects of the high-dose of amphetamine over roughly the first hour of testing, but the 4-way interaction did not reach significance ($p = 0.078$; time course data not shown).

3.3.2.3. Rearing There were no significant main effects or interactions involving prenatal toluene Treatment (p 's > 0.32). A significant main effect was observed for Sex ($F(1,229) = 4.99, p < 0.05$) with males having significantly more rears than females. A significant main effect was observed for amphetamine Dose ($F(2,229) = 21.36, p < 0.001$) with animals treated with amphetamine engaging in more rearing activity than controls. A significant Sex $\times$ amphetamine Dose interaction was also observed ($F(2,229) = 4.15, p < 0.05$) with males displaying more rears than females at the higher dose of amphetamine. (See Figure 5).

4.0 Discussion

We tested the hypothesis that brief, repeated high-concentration *in utero* exposures to toluene produce postnatal behavioral deficits in rats. These results demonstrate for the first time that prenatal toluene exposure can alter sex-influenced postnatal development of spontaneous and amphetamine-induced locomotor activity in rats. These results are particularly important because the binge pattern of toluene exposure that was used models patterns of exposure typical of solvent abuse. Our pregnant rats were exposed 26 times over 13 days to only very brief episodes (15 min, twice daily) of toluene, at high concentrations (8,000 ppm or 12,000 ppm). This exposure protocol seeks to mimic levels achieved during intentional inhalation typical of organic solvent abuse episodes in people, and probably underestimated both the number of exposures and the levels of toluene concentrations achievable in chronic abusers [42,43]. In addition, the impact of prenatal toluene on activity in the present investigation may have been underestimated because the “runts” were culled on PN1 and not included in subsequent analyses of activity. Nevertheless, these “abuse patterns” of gestational (GD8-GD20) toluene exposure resulted in small, significant alterations in spontaneous activity compared to non-exposed rats tested on PN22, but not on PN42 or PN63. In an exploratory secondary analysis, there appeared to be different changes in spontaneous activity as assessed on PN28 in the saline-injected group from Experiment 2. Prenatal exposure to 8,000 ppm toluene also produced a significant hyposensitivity to the locomotor stimulatory effects of an acute amphetamine challenge in male, but not female rats tested on PN28. The current findings also replicate our prior work demonstrating that this high-dose prenatal toluene exposure significantly decreased neonatal body weight, without producing lethal effects in the dams or significant effects on gestation length or litter sex ratio [8].

The effects of *in utero* exposure to relatively lower ambient concentrations of toluene for longer exposure periods, such as those modeling occupational or environmental exposure, have been studied more extensively than abuse exposures. These studies often report no significant impact of toluene exposure. For example, Thiel and Chahoud [46] exposed pregnant Wistar rats to inhaled concentrations of toluene ranging from 100 ppm to 1200 ppm for 6 hrs/day from GD9 to GD21 and reported that even the highest concentration of toluene with this regime did not significantly affect offspring behavior, including coordination on a rotarod at PN18, locomotion at PN31-PN34, and operant discrimination at PN70. Another group of researchers similarly exposed Wistar rats to 1200 ppm toluene for 6 hrs/day from GD7 to PN18 [20] or to 1800 ppm toluene for 6 hrs/day from GD7 to GD20 [23]. These exposures did lower birth weight (consistent with our findings) and induced deficits in surface righting at PN3 and auditory startle at PN13 [20]. However, there were inconsistent effects on open field activity, and no effects on rotarod performance or pre-pulse inhibition of startle [20,23]. There were sex-influenced effects on spatial learning in the Morris maze such that prenatal occupational toluene exposure increased latencies to find the hidden platform during both acquisition and reversal training. These effects were greater in females than males [20,23].

In human inhalant abuse, exposures are rarely if ever continuous as is modeled in most prior prenatal toluene studies using chronic, long-term exposures such as those occupational or environmental exposure models noted above (i.e., > 6 hours exposure per day). Solvent abusers are much more periodic or episodic in their abuse patterns, reportedly inhaling several times a week in brief bouts with many abusers reporting repeated daily use [42,43]. Unfortunately, the previous animal models have varied the temporal pattern of exposure over a restricted range during pregnancy. Compared to continuous lower-dose exposures (e.g., 2,000 ppm for 17 hours), two studies have shown that briefer exposures (60 min) of pregnant mice to higher concentrations of TCE (7,000 ppm to 8,000 ppm) produced similar developmental delays and greater reductions in birth weight and behavioral effects in offspring [12,27]. Jones and Balster [28] approximated a low-level abuse pattern in pregnant mice with inhalations up to 2,000 ppm of toluene limited to three 60-min periods per day for a 5-day period within the human equivalent of mid second trimester (GD12 to GD17). There were small, transient toluene-induced reductions in pup weight, surface righting, inversion righting, and grip strength. In our previous and present studies [8], we used a similar strategy with pregnant rats exposed to repeated 15-min bouts of very high toluene concentrations (8,000 ppm or 12,000 ppm) twice a day from GD8 through GD20 to mimic solvent abuse during pregnancy. Both our previous and present results demonstrated that these high-concentration prenatal toluene exposure patterns can significantly increase the rate of negative postnatal outcome including, for example, decreased body weight and strength, altered negative geotaxis behavior, and increased numbers of minor malformations, while producing little effect on gestation length, or litter sex ratios. These deficits in growth were more persistent after our repeated 15-min binge exposures to high concentrations than were seen with much longer exposures (6 hrs) to lower concentrations (1,800 ppm) in the few other studies with similar prenatal inhalation exposures, even though the apparent total daily exposures were fairly equivalent [8,23,46]. It seems clear that there can be no single optimum model of exposure given the varieties of toluene abuse and we are currently assessing other patterns of exposure.

While the results of this study suggest that the impact of this level of exposure is significant for postnatal outcomes, there appears to be only relatively small changes on spontaneous locomotor activity on any age we tested, including juvenile (PN22 or PN28), “adolescent” (PN42) or young adult (PN63). As noted, there are preliminary indications from our exploratory analysis that the postnatal impact of prenatal toluene exposure may vary in subtle ways with postnatal age of testing. Interestingly, the amphetamine challenge at PN28 appears to reveal a small functional impact of toluene on maturation of monoaminergic systems

supporting locomotor activity that is not evident in spontaneous behavior. While the precise nature of toluene-induced neurotoxicity and its mechanism(s) of action remain to be elucidated, results from previous studies suggest that several systems in addition to the monoamines may be involved, including cholinergic, GABAergic and glutaminergic transmitters [3,4,5,15,33]. Dose-dependent effects of solvent abuse include cerebellar damage [16,17,31,34], white matter abnormalities, such as MRI hyperintensities in cerebrum, thalamus, basal ganglia and cerebellum, and neuronal atrophy of hippocampus, corpus callosum and cerebellar vermis [30,31,37,39,41,42,49,50]. MR imaging of chronic toluene abusers consistently reveals lesions including T-2 hypo- or hyper-intensities throughout brain, including atrophy and white matter changes [30,45,48,49].

Other recent studies have demonstrated that toluene has effects on specific developing neurotransmitter receptors. For example, Cruz et al. [13] showed that toluene nearly eliminated NMDA-stimulated but not kainate-induced currents in *Xenopus laevis* oocytes with low millimolar potency suggesting that NMDA inhibition may be a common mechanism of action for abused inhalants. Toluene also produced dose-dependent and reversible increases in GABA_A and glycine receptor functioning in both *Xenopus* oocytes and rat hippocampal slices [7]. Toluene also has a variety of pharmacological inhibitory and/or stimulatory effects on other neurotransmitter and hormonal systems in adult animals that imply similar actions prenatally with persistent maturational consequences [e.g., 2, 4, 18, 51].

In conclusion, the results of this and previous studies demonstrate that rats are sensitive to neurobehavioral teratogenic effects of an abuse pattern of prenatal exposure to toluene. Additional research directed toward further characterization of the pharmacological and behavioral profiles of rats prenatally exposed to toluene, as well as the neural bases for those profiles, is needed.

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Figure 1.

Effects of prenatal 0, 8,000, or 12,000 ppm toluene exposure on rat offspring distance traveled (top panel) and ambulatory time (bottom panel). Mean total distance (top panels; cm ± SEM) or time (bottom panels; secs ± SEM) are shown for 60-min sessions. The '+' indicates where the two toluene-exposed groups tended to be more active than the air-exposed control group.

Figure 2.

Effects of prenatal 0, 8,000, or 12,000 ppm toluene exposure on number of rat offspring rears at PN42 and PN63. Mean total number of rears ($\pm$ SEM) is shown for 60-min sessions. The ‘*’ indicates significantly different from the air-exposed control group.

Figure 3.

Effects of a single amphetamine challenge (0, 0.56, 1.78 mg/kg) on distance traveled in animals exposed prenatally to 0, 8,000, or 12,000 ppm toluene at PN28. Mean total distance (cm $\pm$ SEM) is shown for the 120-min sessions. Males are depicted in the left column (graphs A, B & C) and females are on the right (graphs D, E & F). The '+' indicates where the toluene-exposed group tended to be more (Panel B) or less (Panel C) active than the air-exposed control group.

Figure 4.

Effects of an amphetamine challenge (0, 0.56, 1.78 mg/kg) on ambulatory time in animals exposed prenatally to 0, 8,000, or 12,000 ppm toluene at PN28. Mean total ambulatory time ($\pm$ SEM) is shown for the 120-min sessions. Males are depicted in the left column (graphs A, B & C) and females on the right (graphs D, E & F). The '+' indicates where the toluene-exposed group tended to be more (Panel B) or less (Panel C) active than the air-exposed control group.

Figure 5.

Effects of an amphetamine challenge (0, 0.56, 1.78 mg/kg) on number of rat offspring rears, at PN42 and PN63 only, in animals exposed prenatally to 0, 8,000, or 12,000 ppm toluene. Mean total number of rears ($\pm$ SEM) is shown for the 120-min sessions. Males are depicted in the left column (graphs A, B & C) and females on the right (graphs D, E & F).

TABLE 1

MATERNAL AND LITTER CHARACTERISTICS (means $\pm$ sem)

Level of Toluene:	0 ppm	8,000 ppm	12,000 ppm	p
Experiment 1				
Number of dams (N=99)	36	31	32	--
Maternal weight on GD4 (g)	224.00 $\pm$ 3.09	223.45 $\pm$ 3.59	229.29 $\pm$ 2.59	ns
Maternal weight gain to GD20 (g) ^a	115.45 $\pm$ 3.21	101.63 $\pm$ 3.48	100.96 $\pm$ 3.29	0.003
Percent weight gain	51.84 $\pm$ 1.55	46.17 $\pm$ 2.00	44.30 $\pm$ 1.61	0.01
Gestational Age (days)	22.25 $\pm$ 0.07	22.26 $\pm$ 0.08	22.31 $\pm$ 0.08	ns
Litter size (Range) ^b	11.72 $\pm$ 0.38 (5–15)	11.39 $\pm$ 0.35 (7–15)	12.81 $\pm$ 0.32 (8–16)	0.02
Litter sex ratio (% males)	51.97 $\pm$ 2.10	47.65 $\pm$ 2.60	48.42 $\pm$ 2.33	ns
Mean pup weight on PN1 (g) ^c	7.19 $\pm$ 0.11	7.21 $\pm$ 0.10	6.88 $\pm$ 0.10	0.048
Experiment 2				
Number of dams (N=87)	26	31	30	--
Maternal weight on GD4 (g)	229.42 $\pm$ 4.21	233.19 $\pm$ 3.12	230.65 $\pm$ 2.60	ns
Maternal weight gain to GD20 (g) ^d	112.51 $\pm$ 4.93	100.92 $\pm$ 2.63	101.27 $\pm$ 2.82	0.04
Percent weight gain	49.86 $\pm$ 2.48	43.72 $\pm$ 1.44	44.15 $\pm$ 1.40	0.03
Gestational Age (days)	22.19 $\pm$ 0.08	22.29 $\pm$ 0.08	22.27 $\pm$ 0.08	ns
Litter size (Range)	12.19 $\pm$ 0.49 (4–16)	12.16 $\pm$ 0.32 (8–16)	12.77 $\pm$ 0.35 (7–16)	ns
Litter sex ratio (% males)	54.40 $\pm$ 2.50	49.45 $\pm$ 2.83	49.53 $\pm$ 2.77	ns
Mean overall weight on PN1 (g)	7.19 $\pm$ 0.13	7.24 $\pm$ 0.11	6.89 $\pm$ 0.12	0.05

^aThe 0 ppm dams gained significantly more weight during gestation than did either of the other toluene-exposed groups. Weight gain was calculated as maternal weight before exposure on GD4 subtracted from maternal weight before exposure on day 20.

^bThere were more pups born in the 12000-ppm group than in the 8,000-ppm group.

^cOn PN1, collapsed on Sex, the 12,000 ppm pups weighed significantly less than did the pups from the other groups, which were similar to each other.

^dThe 0 ppm dams gained significantly more weight during gestation than did either of the other toluene-exposed groups. Weight gain was calculated as maternal weight before exposure on GD4 subtracted from maternal weight before exposure on day 20.