Published in final edited form as:

Neuroscience. 2017 February 07; 342: 180–187. doi:10.1016/j.neuroscience.2015.08.050.

Prenatal Toluene Exposure Impairs Performance in the Morris Water Maze in Adolescent Rats

Sean P. Callan^{a,b}, John H. Hannigan^{a,c,d}, and Scott E. Bowen^{a,b,*}

^aDepartment of Psychology, Wayne State University, Detroit, MI

^bBehavioral Pharmacology and Toxicology Laboratory, Wayne State University, Detroit, MI

^cDepartment of Obstetrics & Gynecology, Wayne State University, Detroit, MI

^dMerrill Palmer Skillman Institute for Child and Family Development, Wayne State University, Detroit, MI

Abstract

Volatile organic solvent abuse continues to be a worldwide health problem, including the neurobehavioral teratogenic sequelae of toluene abuse during pregnancy. Although abuse levels of prenatal toluene exposure can lead to a Fetal Solvent Syndrome, there is little research examining these effects on memory. Consumption of toluene can have detrimental effects on the developing hippocampus which could lead to specific spatial learning and memory deficits. This study used a rat model to determine how prenatal exposure to abuse levels of toluene would affect performance in a spatial learning and memory task, the Morris Water Maze (MWM). Pregnant Sprague-Dawley rats were exposed to 0, 8000 or 12,000 parts per million (ppm) of toluene for 15 min twice daily from gestation day 8 (GD8) through GD20. Male and female offspring (N= 104) were observed in the MWM for 5 days beginning on postnatal day (PN) 28 and again on PN44. While prenatal toluene exposed animals did not differ in initial acquisition in the MWM, rats prenatally exposed to 12,000 ppm toluene displayed performance deficits during a probe trial and in reversal learning on PN44. Overall, this study indicates that prenatal exposure to repeated inhaled abuse patterns of high concentrations of toluene can impair spatial memory function that persists into adolescence.

Keywords

Organic Solvents (Toluene); Prenatal exposures; Spatial Learning and Memory; Rats

INTRODUCTION

The misuse of volatile solvents as intoxicants is a continuing international public health concern (Cruz, 2011, Howard et al., 2011, Bowen and Cruz, 2012). Toluene is among the

*Address correspondence to: S.E. Bowen, Department of Psychology, Wayne State University, 5057 Woodward Avenue, Detroit MI 48202, Scott.Bowen@wayne.edu, Phone: (313) 577-9546, FAX: (313) 577-7636.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

most prevalent and commonly abused solvents in the world. Millions of tons of toluene are produced per year for commercial and industrial use and as such, toluene is found in thousands of household products ranging from glues to paint thinner to gasoline (IARC, 1989, ATSDR, 2000). Toluene is most commonly abused by adolescents with 2.2% of eighth graders in 2013 admitting to deliberately inhaling a solvent in the last 30 days with the intent of getting “high” (Johnston et al., 2015). For 2013, 6.3% of teens in the United States (average age of 19.2 years) reported that an inhalant was the first illicit drug they had ever used with 563,000 people aged 12 or older reporting that they had used an inhalant for the first time in the past year which was only slightly lower than the 2012 estimate of 584,000 people (SAMHSA, 2014). Trends in use in another survey since 1995 are generally decreasing, although in 2013, 8.9% of students reported some form of inhalant abuse at some point during their lives (Kann et al., 2014). While abuse was once predominantly limited to adolescent and pre-adolescent males, the mean age of solvent abuse is increasing and the gap between males and females is now reversed: the percentage of high schools girls reporting that had “ever used inhalants” is now 10% compared to 7.9% for boys (SAMHSA, 2007, Butland et al., 2012, Kann et al., 2014). There is also evidence that a subset of inhalant abusers continue to do so into adulthood (Williams et al., 2007, SAMHSA, 2014). This means that women are at increasing risk of toluene abuse while pregnant.

Toluene is highly lipophilic and inhaled toluene vapor can readily absorb into maternal blood, cross the placenta and reach the blood of developing fetuses. Abuse of inhalants like toluene while pregnant can lead to a constellation of physical anomalies and developmental disabilities in the offspring collectively referred to as “Fetal Solvent Syndrome” (FSS; Toutant and Lippmann, 1979, Hannigan and Bowen, 2010, Bowen and Hannigan, 2013). The signs of FSS in children include premature birth, low birth weight (Ahmed and Jaakkola, 2007), skeletal and other facial anomalies (Jones and Balster, 1998), and impaired cognitive development (Laslo-Baker et al., 2004), outcomes sharing similarities with Fetal Alcohol Spectrum Disorders (FASD; Pearson et al., 1994, Wilkins-Haug, 1997).

Rats exposed prenatally to repeated abuse levels of toluene display similar outcomes, including the gross skeletal abnormalities (Bowen et al., 2009) and behavioral disruptions (Batis et al., 2010, Bowen and Hannigan, 2013). The patterns of behavioral effects of toluene may also parallel those of prenatal alcohol exposure that are mediated by alterations in hippocampal function, including disruptions in spatial performance (Berman and Hannigan, 2000, Brolese et al., 2014, Elibol-Can et al., 2014). The well-established Morris Water Maze task (MWM; Morris, 1984) is used frequently for spatial memory research with rodents (Morris, 1984, Vorhees and Williams, 2006). Deviations in MWM task acquisition have been used as indicators of hippocampal dysfunction (Vorhees and Williams, 2006), and other brain regions involved with planning and executive function (e.g., basal forebrain; D’Hooge and De Deyn, 2001).

Previously published works examining the effects of prenatal toluene exposure on MWM performance had utilized relatively lower toluene concentrations for longer periods of time (e.g., 6 hrs per day) and assessed animals in adulthood (Hass et al., 1999, Hougaard et al., 1999, Hougaard et al., 2005). Hass and colleagues found that prenatal exposure to 1200 ppm toluene impaired the performance of female rats in a re-learning task, but not during initial

learning or during a reversal task (Hass et al., 1999). Hougaard et al. (1999) reported that prenatal exposure to 1800 ppm toluene impaired performance during a reversal task in adult rats, while these same investigators later reported no effect of prenatal exposure to 1500 ppm toluene (with or without the addition of chronic mild stress) on MWM performance in adult rats (Hass et al., 1999, Hougaard et al., 1999, Hougaard et al., 2005). In all cases, testing was carried out on adult offspring following maternal low-concentration exposures to toluene vapor for 6 hrs per day during most of the last two weeks of gestation. We are aware of no prior study assessing the effects of repeated prenatal binge exposure to abuse-levels of toluene on adolescent offspring memory performance. The present study reports the effects of prenatal exposure to relatively higher concentrations of toluene on spatial memory performance in adolescent rats.

EXPERIMENTAL PROCEDURES

Animals

All animal procedures had prior approval by the Wayne State University Institutional Animal Care and Use Committee in accordance with the National Institutes of Health (NIH) “Guide for the Care and use of Laboratory Animals: Eighth Edition” (NRS, 2011). A total of fifty-six timed pregnant Sprague-Dawley rats (200g minimum body weight at mating; ~60 days old) were purchased from Charles Rivers Laboratories (Portage, MI, USA) and arrived on gestation day 4 (GD4; sperm plug positive = GD0). The dams arrived in 5 separate shipments (~10–12 each) and were housed individually in polypropylene cages (52 × 28 × 22 cm) containing wood chip bedding and fitted with steel wire tops. All animals were allowed *ad lib* access to Rodent Lab Diet 5001 (PMI, Nutrition International, Inc., Brentwood, MO) and water while in their home cages but not during the brief toluene exposures. Animals were maintained in an AAALAC-accredited vivarium with temperature controlled to 20–22°C and relative humidity levels between 40% and 70% and a 12-hr light/dark cycle with lights on at 0600 hrs.

Beginning on GD20, dams were inspected frequently between 0800 and 2000 hrs for births with the day of birth designated as postnatal day 0 (PN0), such that the maximum resolution for gestational length was one half day. On PN1, the pups were weighed and examined for obvious physical abnormalities. Experimenters were blind to exposure condition during culling. Pups with any noticeable physical abnormalities or deemed “runts” were weighed and culled. All litters were then culled pseudo-randomly (keeping 5 males and 5 females when possible) to 10 from the remaining pups. Offspring were housed with their dams until weaning on PN21 when all offspring were uniquely identified by ear punch and re-housed in groups of 2 or 3 same-sex littermates and/or other rats from the same prenatal toluene group. All animals (N=104) were unhandled and remained naïve to the testing procedures until testing at PN28. Two pups from each litter (one male and one female) were utilized in this experiment.

Inhalation Exposure Procedures

Four days following arrival at the vivarium, dams were assigned to one of three gestational treatment – “Dose” – groups, balanced for maternal body weight on GD4. From GD8 to

GD20, dams were weighed every other day and exposed daily via inhalation to either 8,000 ppm ($N=18$) or 12,000 ppm of toluene ($N=21$). An “air-only” control group ($N=17$) received the same placement into the chamber without toluene (0 ppm). For each daily exposure, the rats were transported from the vivarium to the lab in their home cages and placed into one of six identical exposure chambers. Exposure to toluene vapor (0 ppm, 8000 ppm, or 12,000 ppm) occurred twice daily between 0900 hrs and 1400 hrs, 2 hrs apart, 15 min each from GD8 through GD20 to simulate repeated high-dose “binge” exposures seen in human toluene abusers. The exposure apparatus and protocol have been described in detail previously (see Bowen et al., 2005). Briefly, animals were placed into a 36-L cylindrical glass jar with acrylic lids equipped with injection ports, a fan, and a stainless steel mesh box holding filter paper. During toluene exposures, one dam was placed onto a grid floor 20 cm from the bottom and 30 cm from the filter paper in the lid of the chamber. The lid was replaced and a calculated amount of toluene (0.0 mL for air controls, 1.36 mL for 8,000 ppm, 2.05 mL for 12,000 ppm) was injected onto filter paper from which the fan volatilized the solvent.

Toluene vapor concentrations were confirmed periodically by single wavelength-monitoring infrared spectroscopy (Miran 1A, Foxboro Analytical). Levels of toluene vapor remained within 5% of the target concentration throughout exposure. Levels of waste gases (i.e., water vapor and CO_2) were monitored during pilot studies and changes during a 15-min session were negligible. The chambers were cleared of vapor with vacuum hoses and the filter paper was replaced between trials to ensure that residual toluene did not accrue over time. Periodic spectroscopy readings confirmed that toluene was completely evacuated from the chambers before the next animal was exposed. Dams in the 0 ppm-control group were placed in an identical exposure chamber at the same time as the toluene exposure group, but exposed to air only. Each animal was exposed to only one solvent concentration. At the conclusion of the exposure period, dams were removed immediately and returned to their home cages to await the next exposure period with the same procedure repeated 2 hrs later. After both daily exposures, animals were returned to the vivarium in their home cages.

Morris Water Maze: Spatial learning and memory

Spatial learning was assessed in a temperature-controlled room (21° C) containing a Morris water maze consisting of a round metal pool (160-cm diameter, 50-cm depth) filled with 30 cm of water (24±1° C) and divided virtually into four equivalent quadrants with distinct visual cues positioned at four points about the room. A small square 15-cm² platform located in the one quadrant was submerged 2.0 cm below the surface of water made opaque by adding black non-toxic tempera paint (Studio Series, Utrecht Art Supplies, Cranbury, NJ). Rats were trained to locate the hidden platform in 3 trials per day with a 30-min inter-trial interval over 5 consecutive days. Temperature and adequate water depth were assessed prior to each day of testing. For each trial, a rat was placed in the maze in a systematically alternating quadrant and allowed to swim freely until it found and climbed onto the hidden platform. After successfully reaching the hidden platform, the rat was allowed to remain on the platform for 10 secs. If unsuccessful, the trial was ended after 90 sec when the experimenter placed the rat on the platform for 10 secs. At the end of Day 5, the platform was removed and a 60-sec probe trial was conducted. Twelve days later (test Day 17; PN44),

animals were placed back into the maze for a second probe trial, followed by three more trials with the platform relocated to assess reversal learning. Movement through the maze was recorded and quantified using Ethovision® XT video tracking system (Noldus Information Technology, Wageningen, Netherlands). A measure of cumulative distance from the platform was also generated as it has been shown to be a reliable measure of spatial learning in animals (Gallagher and Burchinal, 1993). During probe trials, when the platform was removed, “cumulative distance” refers to the distance from the former location of the platform.

Data Analysis

Latency to find the platform, total distance covered, average swim speed, thigmotaxis, and cumulative distance from the platform (after Gallagher and Burchinal, 1993) were each assessed by a $5 \times 3 \times 3 \times 2$ Repeated Measures ANOVA with Day (1–5) and Trial (1–3) serving as the within-subjects factors, and Dose (0 ppm, 8,000 ppm & 12,000 ppm) and Sex as the between-subjects factors. Performance during the Day 17 (PN44) reversal task was analyzed via a $3 \times 3 \times 2$ Repeated Measures ANOVA with Trial as the within-subjects factor and Dose and Sex as between-subjects factors. Comparisons among dose groups used a Tukey’s *A post-hoc* test. Analyses of the probe trials used 2×3 (Sex by Dose) ANOVAs. Maternal weight at GD8 was assessed with an independent samples t-test, while overall maternal weight gain from GD8 to GD20 was assessed with a Repeated Measures ANOVA with day of weighing serving as the within subjects factor and Dose serving as the between subjects factor. The effect of Dose on litter size was assessed via one-way ANOVA. The effect of Dose on PN1 pup weight was assessed using litter weight via an independent samples t-test. The effect of Sex on PN1 pup weight was assessed using a paired samples t-test comparing summed male and female litter weights. The effect of Dose and Sex on PN28 pup weight was assessed via a 3-way ANOVA and an independent samples t-test, respectively. All statistical analyses were conducted using IBM SPSS version 22.

RESULTS

Maternal and Litter Characteristics

Maternal and litter data for the 56 dams in the three prenatal treatment groups are presented in the top panel of Table 1. Dam weights did not differ among Dose groups on GD8 ($p=0.80$) and there was significant weight gain for dams in all Dose groups from GD8 to GD20, $F(1,52) = 1352.31$, $p<0.001$. There was a significant Day $\times$ Dose interaction for maternal weight gain, $F(2,52) = 8.05$, $p<0.01$. *Post-hoc* analyses revealed that dams exposed to 12,000 ppm toluene gained significantly less weight than the air control or 8,000-ppm groups ($p<0.01$). There was no effect of prenatal toluene exposure on litter size ($p=0.93$).

Pup Body Weights

Significant main effects were found for prenatal toluene Dose on PN1 body weight, $F(2,52) = 3.98$, $p<0.05$; Table 1). *Post-hoc* analysis revealed that the 12,000-ppm pups weighed significantly less than the pups in the 8,000-ppm and 0-ppm groups on PN1 ($p<0.05$). Because of clerical error, body weight data are missing for 27 of the 104 pups on PN28 but there were no differences among the Dose groups in missing data. By PN28, there were no

significant differences in litter mean body weight among the toluene Dose groups ($p=0.58$). Male pups weighed significantly more than female pups regardless of Dose at PN1, $t(52) = 5.86$, $p<0.01$, and at PN28, $t(75) = 2.84$, $p<0.01$.

Initial Learning: Latency to platform

For the 104 rat offspring used for the Morris water-maze (MWM) experiments, none had any observable physical abnormalities and all were able to walk and swim without noticeable difficulties. Results from the MWM revealed that all prenatal toluene Dose groups found the platform more quickly across Trials, $F(2, 784) = 24.25$, $p<0.001$, and Days, $F(4, 784) = 69.79$, $p<0.001$ (see Fig. 1A & 1B). Rats exposed prenatally to toluene displayed no differences in latencies to find the platform from non-exposed controls ($p>0.05$). The Trial x Dose interaction approached, but did not reach statistical significance ($p=0.06$), with toluene-exposed animals showing less improvement across trials.

Initial Learning: Speed

Rats that were prenatally exposed to toluene displayed no differences in speed as compared to control rats ($p=0.116$). Swim speed did not differ between Trials, $F(1.00, 100.11) = 3.65$, $p=0.058$, or Days, $F(1.04, 100.11) = 0.67$, $p=0.42$, respectively (data not shown).

Initial Learning: Thigmotaxis

Rats exposed prenatally to toluene displayed no differences in thigmotaxis, defined as time swimming along the edge of the maze next to the wall, as compared to control rats ($p=0.56$). The animals displayed less thigmotaxis in later Trials, $F(1.87, 596.92) = 66.65$, $p<0.001$, and Days, $F(3.25, 596.92) = 109.94$, $p<0.001$. There was a significant effect of Sex, $F(1, 98) = 4.24$, $p<0.05$, with female rats showing increased thigmotaxis compared to males (data not shown).

Initial Learning: Cumulative distance from target

Rats that were prenatally exposed to toluene displayed no differences in cumulative distance from the target compared to 0-ppm control rats ($p=0.46$). Animals displayed smaller average distance from the platform on later Trials, $F(2, 776) = 11.08$, $p<0.001$, and Days $F(4, 776) = 48.66$, $p<0.001$ (data not shown).

Memory: Probe trials

Probe trials (without a platform) were run on test Day 5 (PN32) and Day 17 (PN44) of testing. For the probe trial on Day 5, there was no effect of prenatal toluene Dose on cumulative distance from the platform or on thigmotaxis (p 's = 0.41 & 0.42; 2A). There were no sex differences on cumulative distance from the platform or thigmotaxis ($p = 0.058$ and $p = 0.057$, respectively). For the probe trial on test Day 17, rats prenatally exposed to toluene had significantly greater distances from the platform as compared to the 0-ppm control group, (Dose: $F(2, 98) = 3.3$, $p<0.05$; see Fig 2B). *Post-hoc* analyses reveal that the 12,000-ppm Dose group showed significantly greater cumulative distance from the original platform location during the probe trial compared to air controls ($p<0.05$), while the 8,000-ppm-group displaying non-significant increase over controls ($p=0.13$). There was no effect

of Sex on cumulative distance from the platform ($p=0.90$) or thigmotaxis ($p=0.15$). Finally, there was no effect of prenatal toluene Dose on thigmotaxis on the Day 17 probe trial ($p=0.32$).

Reversal Learning

Rats prenatally exposed to toluene took longer than controls to find the platform during reversal learning on Day 17, $F(2,97) = 3.90$, $p<0.05$ (see Fig. 3). *Post-hoc* tests revealed that the 12,000-ppm toluene Dose group had significantly longer probe trial latencies than control animals. Animals exposed prenatally to toluene also displayed significantly elevated cumulative distances from the platform, $F(2, 98) = 3.16$, $p<0.05$. *Post-hoc* tests revealed that the 12,000-ppm toluene Dose group had significantly elevated cumulative distances from the platform as compared to air controls (Fig. 3C). There were no other significant differences of prenatal toluene exposure in thigmotaxis or swimming speed (p 's =0.14 & 0.20, respectively; data not shown).

DISCUSSION

The present study utilized the Morris Water Maze (MWM) to identify cognitive impairments in adolescent rats that had been exposed prenatally to repeated patterns of high levels of toluene similar to exposures reported in organic solvent abuse. All prenatal toluene Dose groups showed significant learning, as latencies to find the goal platform decreased across Days and Trials. Animals exposed prenatally to 12,000 ppm toluene, however, displayed impaired performance during both the reversal learning phase, improving less from trial-to-trial than animals in other groups, and during the second probe trial 2 weeks after acquisition (on test Day 17/PN44) but not in the first probe trial (Day 5/PN32) that followed immediately after acquisition. These behavioral effects suggest that prenatal toluene exposure impairs longer term spatial memory rather than learning or shorter term recall. The animals exposed to toluene *in utero* were able to learn the MWM task as well as the air-control animals, and when tested with a probe trial on Day 5 did not differ from controls in cumulative distance from the platform location, indicating that both initial spatial learning and memory were intact. However, 12 days later, animals in the 12,000-ppm prenatal toluene exposure condition swam further away from the training location of the goal platform than other groups during the Day 17 probe trial on PN44. This suggests that the 12,000-ppm animals' performance reflected poorer recall than the animals in lower-level exposure groups. While the present data may not exclude errors in consolidation, the Day 17 probe trial on PN44 data suggest that the impairment was in retrieval or recall of a spatial memory and not of a previously-learned search strategy. It is also noted that the performance differences on the probe trial and in reversal learning observed during the later testing on Day 17 tasks did not extend to thigmotaxis, suggesting that animals exposed prenatally to 12,000 ppm toluene were as aware as other groups of the task objective of reaching a goal platform, but were deficient in locating the platform, consistent with a specific spatial memory impairment. The present experimental design cannot rule out the effect of continued maturation on the observed deficits at PN44. It is possible that the toluene-induced learning impairments displayed on PN44 may be the result of cognitive deficits that emerged as maturation progressed, and may not have been present during initial learning

during adolescence. Future studies should seek to experimentally tease apart the effect of maturation from long-term memory ability.

The reduced weight gain in dams after exposure to 12,000-ppm toluene vapor, similar to one of our previous reports (Bowen & Hannigan, 2013), suggests that a toluene-induced maternal toxicity or under-nutrition during pregnancy may have contributed to the current growth restrictions observed in pups at PN1. On the other hand, in another prior study using identical toluene exposures in pregnant rats we reported significant reductions in fetal growth without effects on maternal weight gain (Bowen et al., 2009). However, the lack of significant weight differences in the present investigation at PN28 indicates an increase in relative weight gain over the first 3 weeks of postnatal life for both toluene-exposed groups regardless of any potential maternal toxicity or under-nutrition suggesting that lactation and/or maternal behavior was sufficient for postnatal maturation of the offspring. Indeed, evidence suggests that weight “catch up” can also be beneficial for memory function (Hermann et al., 2009). However, other studies have suggested that animals that have experienced “catch up” growth have reduced dentate granule cell counts, which could play a role in memory function (Bedi, 1991). The effects observed here, however, indicate a dysfunction of long-term memory usage, not memory formation, so we conclude that the prenatal toluene exposure itself is still the most likely cause of the observed effect.

Although previous studies assessing MWM performance following prenatal toluene exposure used much lower concentrations of toluene (1,200 ppm to 1,800 ppm) for longer periods (6 hrs/day) and tested animals as adults (Hass et al., 1999, Hougaard et al., 1999, Hougaard et al., 2005), there are consistencies among outcomes in these and the present work. The prior studies also reported little-to-no effects of prenatal toluene exposure on initial acquisition of the MWM task, and also reported significant effects on reversal or new learning when the platform was moved within the maze tank (Hass et al., 1999, Hougaard et al., 2005). While the present results found no interaction between prenatal toluene exposure and sex on spatial memory performance in the MWM, the prior studies with lower and longer toluene exposures reported conflicting results with adult female rats. One study reported significant effects in females but not males after low-concentration exposures (Hougaard et al., 1999), whereas another study using the same exposure protocol that tested only later adolescent female rats beginning at PN35 found no significant differences in latency at any phase of testing due to prenatal toluene exposure (Ladefoged et al., 2004). However, longer-term deficits in recall were found neither in female offspring following lower-level toluene exposures (Ladefoged, et al., 2004), nor in either sex in the present binge exposure model. While there were no significant interactions with sex in the present study, the previous results may indicate subtle sex differences in initial sensitivity to the effects of prenatal toluene. In general, the present and prior studies together show that prenatal toluene exposure does not appear to impair the initial learning of the MWM task. However, differences in later probe trial performance and in the more challenging reversal or new learning phases of the task when the goal platform is moved suggest modest longer-term spatial memory deficits.

The effects of early developmental toluene exposure on hippocampus-dependent spatial memory performance are consistent with some prior findings on the effects of toluene on the

developing hippocampus, including disrupted synaptogenesis (Lin et al., 2009), increases in NMDA receptors (Lee et al., 2005), and altered expression of neurotrophic factors (e.g., nerve growth factor & brain-derived neurotrophic factor), pro-inflammatory cytokines, and glial markers (e.g. glial fibrillary acidic protein; Win-Shwe et al., 2012). However, while the effects we report are consistent with hippocampal involvement (Morris et al., 1982), other brain areas are also likely to be involved in the behavioral effects of prenatal toluene exposure (D’Hooge and De Deyn, 2001). Interestingly, a study that reported no effects of prenatal toluene exposure on MWM performance also reported no differences in neurochemical indices of neuronal degeneration in the hippocampus (Ladefoged, et al., 2004). Prior work has also demonstrated that prenatal toluene exposure leads to lasting forebrain demyelination, which may also be related to impairments in cognitive flexibility and learning ability (Gospe and Zhou, 1998). Evidence from prior work has implicated the third trimester as critical for toluene-induced hippocampal dysregulation (Win-Shwe and Fujimaki, 2010), while this present model utilized an exposure window analogous with late first and second trimester exposure. It is worth noting that the authors of the prior study used an industrial exposure paradigm (persistent low concentrations of toluene), which may require third trimester exposure to impair memory function. Indeed, prior work has demonstrated that the deleterious effects of toluene exposure are dependent on “dose”, which in inhalation models is a function of concentration and frequency and duration of exposure, with shorter, higher-concentration exposures producing the most profound effects (Jarosz et al., 2008, Callan et al., 2015).

CONCLUSIONS

The present results indicate that prenatal toluene exposures that mimic the high concentrations and repeated, binge patterns of use typical of organic solvent abuse, produce modest impairments in spatial memory in adolescent rats. The results are consistent with some studies using a rat model of “occupational” exposures although the sex differences among studies are not consistent. The results imply that prenatal toluene exposure impairs long-term spatial memory, but not initial learning ability. Future studies should examine the effects of prenatal vs neonatal exposure to illuminate any differences in memory impairment that may arise as a function of when toluene exposure occurs during early brain development. Additionally, future studies should more directly examine the role of hippocampal dysfunction in the behavioral effects observed in this report to determine the mechanism(s) by which toluene impairs memory performance.

Acknowledgments

This work was supported in part by grant R01-DA015951 from the US National Institutes of Health to S.E. Bowen. The contributions of J.C. Batis and M.H. Mohammadi to this study are gratefully acknowledged.

References

- Ahmed P, Jaakkola JJ. Exposure to organic solvents and adverse pregnancy outcomes. *Hum Reprod.* 2007; 22:2751–2757. [PubMed: 17725989]
- ATSDR. Agency for Toxic Substances and Disease Registry, U. S. D. o. H. a. H. S. Toxicological profile for toluene. Atlanta: U.S. Public Health Service; 2000. p. 1-357.

- Batis JC, Hannigan JH, Bowen SE. Differential effects of inhaled toluene on locomotor activity in adolescent and adult rats. *Pharmacology, biochemistry, and behavior*. 2010; 96:438–448.
- Bedi KS. Effects of undernutrition during early life on granule cell numbers in the rat dentate gyrus. *J Comp Neurol*. 1991; 311:425–433. [PubMed: 1955588]
- Berman RF, Hannigan JH. Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus*. 2000; 10:94–110. [PubMed: 10706221]
- Bowen SE, Batis JC, Mohammadi MH, Hannigan JH. Abuse pattern of gestational toluene exposure and early postnatal development in rats. *Neurotoxicol Teratol*. 2005; 27:105–116. [PubMed: 15681125]
- Bowen, SE.; Cruz, SL. Inhalants: addiction and toxic effects in the human. In: Madras, B.; Kuhar, M., editors. *The effects of drug abuse on the human nervous system*. 2012.
- Bowen SE, Hannigan JH. Binge toluene exposure in pregnancy and pre-weaning developmental consequences in rats. *Neurotoxicol Teratol*. 2013; 38:29–35. [PubMed: 23597557]
- Bowen SE, Irtenkauf S, Hannigan JH, Stefanski AL. Alterations in rat fetal morphology following abuse patterns of toluene exposure. *Reproductive toxicology* (Elmsford, NY). 2009; 27:161–169.
- Brolese G, Lunardi P, Broetto N, Engelke DS, Lirio F, Batassini C, Tramontina AC, Goncalves CA. Moderate prenatal alcohol exposure alters behavior and neuroglial parameters in adolescent rats. *Behavioural brain research*. 2014; 269:175–184. [PubMed: 24786333]
- Butland BK, Field-Smith ME, Ramsey JD, Anderson HR. Twenty-five years of volatile substance abuse mortality: a national mortality surveillance programme. *Addiction*. 2012
- Callan SP, Kott JM, Cleary JP, McCarthy MK, Baltes BB, Bowen SE. Changes in Developmental Body Weight as a Function of Toluene Exposure: A Meta-Analysis of Animal Studies. *Human & Experimental Toxicology*. 2015; 1:1–12.
- Cruz SL. The latest evidence in the neuroscience of solvent misuse: an article written for service providers. *Subst Use Misuse*. 2011; 46(Suppl 1):62–67. [PubMed: 21609148]
- D’Hooge R, De Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain research*. 2001; 36:60–90. [PubMed: 11516773]
- Elibol-Can B, Dursun I, Telkes I, Kilic E, Canan S, Jakubowska-Dogru E. Examination of age-dependent effects of fetal ethanol exposure on behavior, hippocampal cell counts, and doublecortin immunoreactivity in rats. *Developmental neurobiology*. 2014; 74:498–513. [PubMed: 24302592]
- Gallagher MBR, Burchinal M. Severity of Spatial Learning Impairment in Aging: Development of a Learning Index for Performance in the Morris Water Maze. *Behavioral Neuroscience*. 1993; 107:618–626. [PubMed: 8397866]
- Gospe SM Jr, Zhou SS. Toluene abuse embryopathy: longitudinal neurodevelopmental effects of prenatal exposure to toluene in rats. *Reproductive toxicology* (Elmsford, NY). 1998; 12:119–126.
- Hannigan JH, Bowen SE. Reproductive toxicology and teratology of abused toluene. *Syst Biol Reprod Med*. 2010; 56:184–200. [PubMed: 20377315]
- Hass U, Lund SP, Hougaard KS, Simonsen L. Developmental neurotoxicity after toluene inhalation exposure in rats. *Neurotoxicol Teratol*. 1999; 21:349–357. [PubMed: 10440478]
- Hermann GM, Miller RL, Erkonen GE, Dallas LM, Hsu E, Zhu V, Roghair RD. Neonatal catch up growth increases diabetes susceptibility but improves behavioral and cardiovascular outcomes of low birth weight male mice. *Ped Res*. 2009; 66:53–58.
- Hougaard KS, Andersen MB, Hansen AM, Hass U, Werge T, Lund SP. Effects of prenatal exposure to chronic mild stress and toluene in rats. *Neurotoxicol Teratol*. 2005; 27:153–167. [PubMed: 15681129]
- Hougaard KS, Hass U, Lund SP, Simonsen L. Effects of prenatal exposure to toluene on postnatal development and behavior in rats. *Neurotoxicol Teratol*. 1999; 21:241–250. [PubMed: 10386827]
- Howard MO, Bowen SE, Garland EL, Perron BE, Vaughn MG. Inhalant use and inhalant use disorders in the United States. *Addiction science & clinical practice*. 2011; 6:18–31. [PubMed: 22003419]
- IARC. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Organic Solvents, Resin Monomers and Related Compounds, Pigment and Occupational Exposures in Paint Manufacturing and Painting. Vol. 47. Lyon, France: International Agency for Research on Cancer, World Health Organization; 1989.

- Jarosz PA, Fata E, Bowen SE, Jen KL, Coscina DV. Effects of abuse pattern of gestational toluene exposure on metabolism, feeding and body composition. *Physiology & behavior*. 2008; 93:984–993. [PubMed: 18272189]
- Johnston, LD.; O'Malley, PM.; Miech, RA.; Bachman, JG.; Schulenberg, JE. Monitoring the Future national survey results on drug use: 1975–2014: Overview, key findings on adolescent drug use. Ann Arbor: Institute for Social Research, The University of Michigan; 2015.
- Jones HE, Balster RL. Inhalant abuse in pregnancy. *Obstetrics and gynecology clinics of North America*. 1998; 25:153–167. [PubMed: 9547765]
- Kann L, Kinchen S, Shanklin SL, Flint KH, Kawkins J, Harris WA, Lowry R, Olsen EO, McManus T, Chyen D, Whittle L, Taylor E, Demissie Z, Brener N, Thornton J, Moore J, Zaza S. Centers for Disease C and Prevention . Youth risk behavior surveillance--United States, 2013. Morbidity and mortality weekly report Surveillance summaries. 2014; 63(Suppl 4):1–168.
- Ladefoged O, Hougaard KS, Hass U, Sorensen IK, Lund SP, Svendsen GW, Lam HR. Effects of combined prenatal stress and toluene exposure on apoptotic neurodegeneration in cerebellum and hippocampus of rats. *Basic Clin Pharmacol Toxicol*. 2004; 94:169–176. [PubMed: 15078341]
- Laslo-Baker D, Barrera M, Knittel-Keren D, Kozer E, Wolpin J, Khattak S, Hackman R, Rovet J, Koren G. Child neurodevelopmental outcome and maternal occupational exposure to solvents. *Arch Pediatr Adolesc Med*. 2004; 158:956–961. [PubMed: 15466682]
- Lee YF, Lo PS, Wang YJ, Hu A, Chen HH. Neonatal toluene exposure alters N-methyl-D-aspartate receptor subunit expression in the hippocampus and cerebellum in juvenile rats. *Neuropharmacology*. 2005; 48:195–203. [PubMed: 15695158]
- Lin H, Liu C, Jow G, Tang C. Toluene disrupts synaptogenesis in cultured hippocampal neurons. *Toxicology letters*. 2009; 184:90–96. [PubMed: 19038319]
- Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of neuroscience methods*. 1984; 11:47–60. [PubMed: 6471907]
- Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. *Nature*. 1982; 297:681–683. [PubMed: 7088155]
- NRS. Guide for the care and use of laboratory animals. Washington, D.C: National Research Council (U.S.), Institute for Laboratory Animal Research (U.S.), National Academies Press; 2011. p. xxvp. 220
- Pearson MA, Hoyme HE, Seaver LH, Rimsza ME. Toluene embryopathy: delineation of the phenotype and comparison with fetal alcohol syndrome. *Pediatrics*. 1994; 93:211–215. [PubMed: 7510061]
- SAMHSA. The NSDUH Report March 15, 2007 Patterns and Trends in Inhalant Use by Adolescent Males and Females: 2002–2005. Substance Abuse and Mental Health Services Administration, U.S. Department of Health and Human Services; 2007. No. NSDUH07-0315
- SAMHSA. Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings. Rockville, MD: Substance Abuse and Mental Health Services Administration; 2014. NSDUH Series H-48, HHS Publication No. (SMA) 14-4863
- Toutant C, Lippmann S. Fetal solvents syndrome. *Lancet*. 1979; 1:1356. [PubMed: 87825]
- Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nature protocols*. 2006; 1:848–858. [PubMed: 17406317]
- Wilkins-Haug L. Teratogen update: toluene. *Teratology*. 1997; 55:145–151. [PubMed: 9143096]
- Williams JF, Storck M. American Academy of Pediatrics Committee on Substance Abuse, American Academy of Pediatrics Committee on Native American Child Health . Inhalant abuse. *Pediatrics*. 2007; 119:1009–1017. [PubMed: 17473104]
- Win-Shwe TT, Fujimaki H. Neurotoxicity of toluene. *Toxicology letters*. 2010; 198:93–99. [PubMed: 20599484]
- Win-Shwe TT, Kunugita N, Yoshida Y, Nakajima D, Tsukahara S, Fujimaki H. Differential mRNA expression of neuroimmune markers in the hippocampus of infant mice following toluene exposure during brain developmental period. *J Appl Toxicol*. 2012; 32:126–134. [PubMed: 21381054]

Highlights

- 1.** Prenatal toluene exposure impairs longer-term reversal learning in rats in the Morris Water Maze.
- 2.** Prenatal toluene exposure impairs long term, but not immediate, water maze Probe trial performance.
- 3.** Solvent abuse during pregnancy potentially has life-long implication for behavior and cognitive functioning in offspring.

Figure 1. Spatial learning in the fixed-platform water maze task

Shown are the effects of prenatal toluene exposure (0, 8000 ppm or 12000 ppm; $n=32-37$ per treatment) on latency to locate the platform by (A) Trial and (B) Day during initial learning. There were significant effects of Trial and Day with all animals showing a reduction in time to escape over the 3 trials and the 5 days. There were no significant effects of Sex or prenatal toluene Dose, or interactions between these factors. The Trial $\times$ Dose interaction approached but did not reach statistical significance (panel A; $p = 0.06$). Means $\pm$ sem.

Figure 2. Probe Trials

Shown are the effects of prenatal toluene exposure (0, 8000 ppm or 12000 ppm; $n=32-37$ per treatment) on cumulative distance from the training platform location on training (A) Day 5 and (B) Day 17. (A) There were no significant effects of Dose on Day 5. (B) On Day 17, there was a significant effect of Dose with 12,000-ppm toluene animals showing greater cumulative distances from the platform (means $\pm$ sem; * $p < 0.05$).

Figure 3. Reversal Learning

Shown are the effects of prenatal toluene exposure (0, 8000 ppm or 12,000 ppm; n=32–37 per treatment) on latency to reach the platform during the reversal learning experiment. (A) mean latency across all three trials; (B) mean latency for each trial individually; and (C) mean cumulative distance traveled. 12,000 ppm toluene animals showed longer latencies and distance traveled to find the platform (means±sem; * $p<0.05$).

TABLE 1

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

Level of Toluene:	0 ppm	8,000 ppm	12,000 ppm	<i>p</i>
Number of dams (<i>N</i> =56)	17	18	21	--
Maternal weight on GD8 (g)	244.60 $\pm$ 5.34	240.55 $\pm$ 4.45	241.08 $\pm$ 3.87	ns
Maternal weight gain to GD20 (g)	98.98 $\pm$ 4.94	85.49 $\pm$ 3.91	76.27 $\pm$ 3.29 ^{<i>a</i>}	0.001
Litter size (range)	12.17 $\pm$ 0.58 (8–16)	12.16 $\pm$ 0.41 (9–15)	11.95 $\pm$ 0.49 (8–17)	ns
Litter sex ratio (% males)	53.32 $\pm$ 5.50	49.11 $\pm$ 2.30	43.41 $\pm$ 3.73	ns
Overall weight on PN1 (g)	7.21 $\pm$ 0.21	7.01 $\pm$ 0.10	6.69 $\pm$ 0.09 ^{<i>b</i>}	0.05
Male weight on PN1 (g)	7.32 $\pm$ 0.24	7.16 $\pm$ 0.11	7.08 $\pm$ 0.16	ns
Female weight on PN1 (g)	6.96 $\pm$ 0.20	6.77 $\pm$ 0.10	6.54 $\pm$ 0.10	ns
Overall weight on PN28 (g)	85.34 $\pm$ 1.50	85.01 $\pm$ 1.42	83.09 $\pm$ 1.10	ns
Male weight on PN28 (g)	89.18 $\pm$ 1.84	87.63 $\pm$ 1.56	84.43 $\pm$ 1.79	ns
Female weight on PN28 (g)	82.48 $\pm$ 1.99	82.57 $\pm$ 2.17	81.67 $\pm$ 1.20	ns

^{*a*}The dams in the 12,000-ppm Dose group gained significantly less weight than dams in the 0-ppm air control group. Weight gain was calculated as maternal weight before exposure on GD4 subtracted from maternal weight before exposure on GD20.

^{*b*}Pups in the 12,000-ppm group weighed significantly less at birth than pups in the 0-ppm group.