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PRENATAL TOLUENE EXPOSURE IMPAIRS PERFORMANCE IN THE MORRIS WATER MAZE IN ADOLESCENT RATS

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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 ppm (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.

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Key words: 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 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 preadolescent males, the mean age of solvent abuse is increasing and the gap between males and females is now reversed: the percentage of high school 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 and Storck, 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 be absorbed 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).

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Abbreviations: FSS, Fetal Solvent Syndrome; GD, gestation day; MWM, Morris Water Maze; PN, postnatal day.

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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 low toluene concentrations for longer periods of time (e.g., 6 h per day) and assessed animals in adulthood (Hass et al., 1999; Hougaard et al., 1999, 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, 2005). In all cases, testing was carried out on adult offspring following maternal low-concentration exposures to toluene vapor for 6 h 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 high 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 (200 g 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 five 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, USA) 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-h light/dark cycle with lights on at 06:00 h.

Beginning on GD20, dams were inspected frequently between 08:00 and 20:00 h 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 blinded to exposure conditions during culling. Pups with any noticeable physical abnormalities or deemed "runts" were weighed and culled. All litters were then culled pseudo-randomly (keeping five males and five 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 two or three 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 8000 ppm (N = 18) or 12,000 ppm 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 h and 1400 h, 2 h 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 8000 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

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