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Abstinence following toluene exposure increases anxiety-like behavior in mice☆

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ABSTRACT

The intentional misuse of volatile solvents like toluene is a persistent public health concern. Limited clinical data suggest that chronic inhalant abusers may experience signs of withdrawal, including anxiety. Behavioral withdrawal from toluene has not been examined in a preclinical model. In the current study, young adult male Swiss Webster mice were exposed to either 5000-ppm toluene vapor or air (0 ppm) for 30 min or 24 h. Mice were tested in a battery of four behavioral tasks reflective of anxiety either immediately (0 h), 24 h, or 72 h after the toluene exposure. Mice exposed briefly (30 min) to toluene showed decreases in anxiety-like behaviors, whereas mice abstinent from toluene for 24 h after a prolonged (24-h) exposure, displayed increases in anxiety-like behaviors. These increases in anxiety-like behavior were not observed 72 h post toluene. However, a brief re-exposure to toluene (30 min at 5000 ppm) immediately before testing 24 h after the prolonged exposure ameliorated behavioral differences on the plus maze task. These results of 1) decreased anxiety-like behavior immediately following acute toluene, and 2) the contrasting increase in anxiety-like behavior during abstinence from a prolonged toluene exposure, and 3) the amelioration of increases in an anxiety-like behavior following toluene re-exposure, are consistent with an interpretation of withdrawal from the single 24-hr toluene exposure. These findings support clinical reports of increased anxiety during abstinence following periods of toluene use/abuse. The results also imply that experiencing anxiety during withdrawal from toluene may contribute to the persistent use of inhalants and may be relevant to clinical treatment of inhalant abuse/addiction.

1. Introduction

The deliberate inhalation of organic solvents, such as toluene, is a persistent public health issue. In 2016, > 500,000 people in the United States aged 12 or older reported inhalant use in the past year (SAMHSA, 2016). Despite the high risk of serious harm or even death (Bowen, 2011; Bowen et al., 1999; Butland et al., 2012), inhalants remain one of the most commonly abused drug types in developing countries (Howard et al., 2011). In the United States, inhalant abuse is relatively more prevalent among younger individuals. While abuse among adolescents has been trending downward, it was recently reported that 7.7% of 8th grade students admitted to having used inhalants (Johnston et al., 2017).

Clinical evidence suggests that individuals can develop long-standing patterns of recurring inhalant abuse and experience difficulty in maintaining sobriety (Basu et al., 2004; Narayanaswamy et al., 2012; Quraishi et al., 2013; Verma et al., 2011). Individuals who try to abstain from using inhalants may experience withdrawal symptoms that abate upon relapse (Shah et al., 1999). In a 2017 report from India, 78% of male teens appearing at a clinic to seek treatment for inhalant abuse (esp., ink eraser fluid or glue) experienced signs of withdrawal indicated frequently by anxiety, irritability, fatigue, headache, craving and poor concentration (Bhad et al., 2017). This is true specifically for toluene, one of the most commonly abused inhalants (Evans and Raistrick, 1987a). Toluene abusers also reported symptoms of anxiety, aggression, and tremors beginning shortly after a period of inhalation (Kouzoupis et al., 2010). Together these results suggest that toluene abuse, and likely inhalant abuse in general, produces clinically significant signs of withdrawal in humans.

The small number of clinical studies along with possible confounds

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(e.g., self-selection, self-report, small sample sizes, poor characterization of exposures, poly-substance abuse, etc.) limits the generalizability of findings and limits replication in pre-clinical models. Elucidating the behavioral consequences of inhalant abstinence specific to toluene in a well-controlled experiment is important for understanding withdrawal from abused inhalants. Animal models allow methodological control and ability to investigate the effects of withdrawal from toluene without potential confounds. Yet there are few reports investigating withdrawal from inhalants in animal models. Evans and Balster (1993) found that prolonged exposure to 1,1,1 trichlorethane (TCE) resulted in an increase in transient seizure activity in mice and that re-exposure to TCE (or exposure to toluene) mitigated this effect. However, there have been no published investigations of behavior indicating anxiety or other putative cognitive/affective signs of toluene withdrawal in an animal model.

The present study investigated multiple anxiety-associated behaviors in mice after a 30-min ("acute") toluene exposure and after periods of abstinence following a prolonged 24-h toluene exposure. As in previous studies, we expected that acute toluene inhalation would decrease anxiety-like behaviors. We hypothesized that mice that had been exposed to a prolonged 24-h toluene inhalation would exhibit transient increases in anxiety-like behaviors at 24 h and 72 h after the single, prolonged exposure. Further, in a small follow-up study, we hypothesized that re-exposure to toluene vapor immediately after a 24-h period of abstinence, and before testing, would reduce anxiety-like behavior observed during abstinence, consistent with a hypothesis that a toluene exposure pattern mimicking inhalant abuse would reduce the behavioral signs of withdrawal.

2. Materials and methods

2.1. Subjects

Outbred male Swiss Webster mice ($N = 260$; Envigo RMS, Indianapolis, IN, USA) arrived in the lab at 30 days of age. Mice were housed 10–12 per polypropylene cage (52 × 28 × 22 cm) containing corncob bedding and fitted with steel wire tops in an AAALAC-accredited vivarium. Mice had ad libitum access to water and chow (Rodent Lab Diet 5001, PMI Nutrition International, Inc., Brentwood, MO) in a temperature- (20 °C–22 °C) and humidity-controlled environment (40%–70%) with a 12-h light cycle (0600 h–1800 h). In the experiments involving 24-h toluene exposure, animals were weighed before being placed into the exposure chambers, immediately upon removal after the 24-h exposure, and then at 24 h and/or 72 h after removal from the chamber. The Institutional Animal Care and Use Committee at Wayne State University approved all procedures beforehand. Procedures were conducted in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (NRS, 2011).

2.2. Static exposure chambers

The static solvent exposure system has been described previously in detail (Apawu et al., 2015; Bowen et al., 2010; Conti et al., 2012). Toluene exposures were given in one of 6 sealed 26-l cylindrical glass jars in a fume hood. A mouse was placed individually into the chamber ~38 cm from a vapor diffuser with a fan attached to the Plexiglas lid. An amount of liquid toluene calculated to produce 5000 ppm was placed into the diffuser and the fan volatilized and distributed toluene vapor throughout the chamber. Toluene concentrations were confirmed periodically using single wavelength-monitoring infrared spectrometry (Miran 1A, Foxboro Analytical). Mean concentrations of toluene were within 3% of nominal levels ~2.5 min after toluene was added, and remained within 2% of nominal throughout the 30-min exposure. For the air-only (0 ppm) group, the lid was sealed, nothing was injected into the diffuser, and the fan was turned on for 30 min. Levels of waste gases (i.e., water vapor & CO_2) were monitored periodically and changes were negligible (e.g., mean CO_2 < 900 ppm). After the 30-min exposures, the lids were opened and toluene vented. Toluene concentrations were below detectable levels after ~1 min.

2.3. Dynamic exposure chambers

The dynamic solvent exposure system has been described previously in detail (Bowen and Balster, 1996; Bowen et al., 1998). Briefly, mice in groups of 10 were exposed to toluene vapors in a 37.8-L glass tank (51 cm × 28 cm × 12 cm high) with a tight-fitting Plexiglas® lid. Toluene vapors were generated using a flow regulator which allowed filtered air to pass through a gas dispersion tube immersed in 500-ml of liquid toluene in a 1-L round-bottom flask. Toluene-saturated air was mixed with fresh filtered air delivered to the exposure chamber via a 1.2-cm dia. access port at one end of the lid. By adjusting the toluene-saturated/filtered air ratio and the flow rate through the chamber (approx. 10 L/min), the toluene concentration was held constant at 5000 ppm. Toluene concentration in the chamber was monitored in-line using a single wavelength monitoring infrared (IR) spectrometer (Miran 1A, Foxboro Analytical, North Haven, CT). Animals in an air-control condition were placed in identical chambers but without toluene vapor. Before each exposure session, fresh chow and water were placed into the chambers and the floors were covered with fresh bedding and a 'crinkle nestlet'.

2.4. Toluene exposure groups

The design of these experiments was modeled after a study of withdrawal from TCE exposure by Evans and Balster (1993). However, using a 96-h exposure to toluene proved more toxic than TCE, so exposures were limited to 24 h (addressed further in the Discussion). In the present study, mice were exposed to toluene for either a brief ("acute") 30-min exposure or a prolonged 24-h exposure. In the acute condition (Experiment 1), mice inhaled 5000 ppm toluene (Acute-TOL) or 0 ppm (Acute-AIR) for 30 min and were then removed from the chambers and behavior tested immediately in one of four behavioral tasks (described below) reflecting anxiety. For the prolonged exposure (Experiment 2), separate groups of mice were exposed for 24 h to toluene at either 5000 ppm (TOL) or 0 ppm (AIR). In Experiment 2, separate groups of mice exposed for 24 h were tested on one of the same four behavioral tasks either 24 h or 72 h after removal from the exposure chambers (i.e., after different times of abstinence from the prolonged toluene exposure). In Experiment 3, other mice were given a 24-h exposure to the chambers with either toluene (5000 ppm) or AIR (0 ppm) and a 24-h period of abstinence after removal from the exposure chambers as in Experiment 2, but these mice were then given a brief, 30-min re-exposure to the chamber with toluene in the static exposure chamber (5000 ppm) and tested immediately on the elevated plus maze task. See Fig. 1 for the overall design.

The high 5000-ppm concentration was chosen to model solvent abuse and was based on our prior demonstrations of consistent behavioral effects (Apawu et al., 2015; Bowen et al., 2010). In Experiment 1, there were 10 mice per exposure group (Acute-TOL & Acute-AIR) and for each of the four behavioral tasks detailed below (2 × 4 × 10; $N = 80$ mice). In Experiment 2, there were 10 mice in each group exposed for 24 h to TOL or AIR, and after each period of abstinence (24 h or 72 h), and for each of the four behavioral tasks (2 × 2 × 4 × 10, $N = 160$ mice). During the 24-h toluene exposure period, animals were observed every hour for the first 10 h (1000 h to 2000 h) for any obvious adverse effects. Toluene exposures ended after 24 h when the mice were removed from the chambers and returned to their home cages until behavioral testing began.

In Experiment 3, different groups of mice were placed for 24 h in the exposure chambers with TOL (5000 ppm) or AIR (0 ppm) and then tested in the elevated plus-maze task 24 h later. Immediately before behavioral testing, these mice were given a single 30-min re-exposure