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Chronic intermittent toluene inhalation initiated during adolescence in rats does not alter voluntary consumption of ethanol in adulthood

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A B S T R A C T

Voluntary inhalation of organic solvents, such as toluene, is particularly prevalent in adolescent populations and is considered to be a contributing factor to substance use and dependence later in life. While inhalants are often the initial "drug" experienced during this period, alcohol is another substance readily abused by adolescent populations. Although both substances are thought to have similar actions within the brain, our understanding of the implications of adolescent inhalant abuse upon subsequent exposure to alcohol remains to be investigated. Thus, this study aimed to assess locomotor responses to acute ethanol and voluntary ethanol consumption following a period of toluene inhalation throughout adolescence/early adulthood. Adolescent male Wistar rats (postnatal day [PN] 27) inhaled air or toluene (3000 ppm) for 1 h/day, 3 days/week for 4 (PN 27–52) or 8 weeks (PN 27–80) to mimic the patterns observed in human inhalant abusers. Following the exposure period, cross-sensitization to acute ethanol challenge (0.5 g/kg, intraperitoneally [i.p.]), and voluntary consumption of 20% ethanol in a chronic intermittent 2-bottle choice paradigm, were assessed. Hepatic ethanol and acetaldehyde metabolism and liver histopathology were also investigated. Chronic intermittent toluene (CIT) exposure throughout adolescence for up to 8 weeks did not alter the behavioral response to acute ethanol or voluntary consumption of ethanol in adulthood, although an age-dependent effect on ethanol consumption was observed ($p < 0.05$). Both liver function and pathology did not differ between treatment groups. Thus, in the paradigm employed, CIT exposure throughout adolescence and early adulthood did not predispose rats to subsequent locomotor sensitivity or voluntary consumption of ethanol in adulthood.

Introduction

Inhalation of volatile organic solvents is particularly prevalent among adolescent populations, with inhalants commonly being the initial "drug" experienced (Lubman, Hides, & Yücel, 2006). Accordingly, the incidence of inhalant abuse is highest in adolescents aged between 12 and 18 years of age, with at least 26% of Australian secondary school students having experimented with inhalants (White, 2001), and over 77% continuing to abuse inhalants for periods greater than 1 year (Neumark, Delva, & Anthony, 1998). Furthermore, in the 2010 National Drug Strategy Household Survey conducted by the Australian Government, a 23% increase in inhalant abuse was reported between 2007 and 2010, while both alcohol and tobacco use decreased during this period (AIHW, 2011). Thus, within Australian communities, as in many other countries such as the United States, Mexico, Brazil, and Japan, adolescent inhalant misuse is a significant growing public health concern (for review see Dell, Gust, & MacLean, 2011).

The prevalence of inhalant misuse in adolescence is thought to be due to the fact that they are cheap, legal, readily accessible, and provide a rapid intoxicating effect (Lubman, Yücel, & Lawrence, 2008). Preferentially abused products such as paints, glues, and aerosols contain a variety of volatile compounds, yet it is the volatile organic solvent toluene that is considered to have the highest abuse potential. Indeed, toluene elicits a conditioned place preference in rodents (Funada, Sato, Makino, & Wada, 2002) and is self-administered in mice (Blokhina, Dravolina, Bespalov, Balster, & Zvartau, 2004) and non-human primates (Weiss, Wood, & Macys, 1979). Furthermore, like other drugs of abuse, toluene's ability to regulate signaling within the mesocorticolimbic dopaminergic (DA) system is thought to underlie its acute hedonic and reinforcing

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properties (Beckley, Evins, Fedarovich, Gilstrap, & Woodward, 2013; Riegel, Zapata, Shippenberg, & French, 2007).

Chronic abuse of products containing toluene is associated with both cognitive and neurobiological abnormalities (Dick, Axelsson, Lawrence, & Duncan, 2013; Yücel, Takagi, Walterfang, & Lubman, 2008). Inhalation of such products during adolescence is of particular concern as it has a significant co-morbidity with neuropsychiatric disorders and is a predictor of substance abuse later in life (Wu, Pilowsky, & Schlenger, 2004). The vulnerability of the adolescent brain to substances of abuse is believed to be driven, in part, by the fact that adolescence encompasses a significant maturational period in which dynamic reorganization occurs within the brain (for review see Lubman, Yücel, & Hall, 2007; Spear, 2000). As such, the long-term adverse consequences of inhalant abuse are associated with the age of abuse onset (Wu et al., 2004), as well as the duration of abuse history in humans (Yücel et al., 2008) and rodents (Duncan et al., 2012).

Similarly, alcohol abuse is prevalent in adolescent populations and is a predictor of alcohol use and dependence later in life (Chassin, Pitts, & Prost, 2002). These observations have been supported by rodent models showing adolescent ethanol exposure elicits increased ethanol consumption and/or preference in adulthood (Criado & Ehlers, 2013; Maldonado, Finkbeiner, Alipour, & Kirstein, 2008; Pascual, Boix, Felipo, & Guerri, 2009). Interestingly, like ethanol and other classic central nervous system depressants, toluene exerts biphasic locomotor effects inducing motor excitation at low concentrations (<4000 ppm) and motor impairment, sedation, and anesthesia at high concentrations (>6000 ppm) (Bowen & Balster, 1998). This is thought to be due in part to several neuropharmacological similarities between toluene and ethanol (Cruz, Mirshahi, Thomas, Balster, & Woodward, 1998; Lovinger, White, & Weight, 1990). Toluene inhalation also acts as an ethanol-like discriminative stimulus in rodents, suggestive of a degree of similarity in the hedonic reinforcing properties of these substances (Bowen, 2009; Rees, Knisely, Breen, & Balster, 1987). Thus, due to the similar actions mentioned above, adolescent toluene exposure may also alter subsequent consumption of ethanol or administration of other drugs of abuse in adulthood, although this remains to be investigated.

Consequently, the present study aimed to investigate the effect of CIT exposure at a positively reinforcing concentration (3000 ppm) (Funada et al., 2002) during adolescence and early adulthood upon subsequent ethanol-induced locomotor activity and voluntary ethanol consumption in adulthood. As it has been previously demonstrated that the duration of CIT exposure results in differential neuropathological and behavioral effects in this model (Duncan et al., 2012), this study investigated the effects of ethanol following exposure confined primarily to adolescence (PN 27–52) or both adolescence and early adulthood (PN 27–80). As age-dependent effects on voluntary ethanol consumption have been observed in rats (Schramm-Sapyta et al., 2013), this paradigm also enabled the investigation of the effects of age upon voluntary ethanol consumption. Due to the potential hepatotoxic nature of adolescent CIT exposure, endogenous liver enzyme activity and liver pathology were also assessed. It was hypothesized that CIT exposure for up to 8 weeks would alter both the locomotor response to ethanol and voluntary consumption of ethanol in adulthood, and that the effect of toluene would be related to duration of exposure.

Materials and methods

Animals

Adolescent male Wistar rats (PN 24) were obtained from the Australian Resources Centre (Perth, Australia). In rats, adolescence ranges from weaning at PN 21 to adulthood at PN 60 (for review see Spear, 2000). Rats were pair-housed, maintained on a 12-h light/dark cycle (lights on at 7:00 AM) and given access to food and water *ad libitum*, with 2 water bottles available to reduce novelty in the 2-bottle choice paradigm. Rats were acclimatized for 3 days prior to any experimental manipulation. All experiments were approved in advance by the Florey animal ethics committee and were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

Toluene inhalation exposure

Exposure to toluene inhalation was conducted as previously described (Duncan et al., 2012). Briefly, rats were acclimatized to the laboratory at least 1 h prior to exposure to toluene or air during which time their body weights were recorded. Exposure to vaporized toluene was conducted in specialized chambers (4.76 L; 17.6 × 16.5 × 16.4 cm) connected to a toluene vapor system whereby air was pumped through liquid toluene (1.08389, purity 99.8%, Merck, Vic, Australia) in a gas wash-bottle to produce toluene vapor. Flow meters allowed the regulation of the desired concentration of toluene vapor, which was verified using a previously calibrated inline gas chromatography system (Shimadzu Corporation, Kyoto, Japan). A minimum of 3 readings were taken per session with deviations greater than 100 ppm of the desired toluene concentration being corrected. Chambers of similar design but exposed to room air only were utilized for control animals (0 ppm exposure).

Rats were randomly assigned to inhale either air ($n = 24$) or toluene (3000 ppm, $n = 24$) for 1 h per day, 3 days per week (Monday, Wednesday, Friday), for 4 weeks (air $n = 12$; CIT $n = 12$) or 8 weeks (air $n = 12$; CIT $n = 12$). Thus, the exposure period took animals from early adolescence (PN 27) to late adolescence (PN 52, 4 weeks exposure) or early adulthood (PN 80, 8 weeks exposure). The chronic intermittent paradigm employed was designed to mirror the human pattern of toluene abuse (Lubman et al., 2008). After 1 h of exposure, rats were placed back into their home cages and isolated from other rats for 1 h to avoid the possible confounds of olfactory stimulation by toluene scent on the fur. All chambers were briefly cleaned with 70% ethanol between sessions. Exposures were conducted at room temperature (~21 °C) under normal lighting and each rat was exposed at approximately the same time each day (~9:00–11:00 AM or 2–4 h into the light cycle).

Ethanol cross-sensitization

To test for locomotor cross-sensitization to ethanol following adolescent CIT exposure, air- and CIT-exposed rats were subjected to an acute ethanol challenge. Commencing 3 days following the final air/CIT exposure, rats were habituated to locomotor cells (TruScan™ Photobeam; Coulbourn Instruments, Allentown, PA, USA) over 3 daily habituation sessions, where locomotor activity was monitored for 15 min followed by administration of saline (10 mL/kg, i.p.) and monitoring of locomotor activity for a further 30 min. Following the habituation period, rats were administered ethanol (0.5 g/kg, i.p.) under the same conditions as the saline exposure paradigm (i.e., 15–min habituation, injection and locomotor activity monitored for a further 30 min; PN 61, 4-week cohort; PN 86, 8-week cohort). Horizontal (HP) and vertical plane (VP) activity was recorded throughout all sessions.

2-Bottle choice paradigm

Following ethanol cross-sensitization, rats were single housed, and the effect of adolescent CIT exposure upon voluntary ethanol