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Persistent cognitive and morphological alterations induced by repeated exposure of adolescent rats to the abused inhalant toluene

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ABSTRACT

While the psychoactive inhalant toluene causes behavioral effects similar to those produced by other drugs of abuse, the persistent behavioral and anatomical abnormalities induced by toluene exposure are not well known. To mimic human "binge-like" inhalant intoxication, adolescent, male Sprague-Dawley rats were exposed to toluene vapor (5700 ppm) twice daily for five consecutive days. These rats remained in their home cages until adulthood (P60), when they were trained in operant boxes to respond to a palatable food reward and then challenged with several different cognitive tasks. Rats that experienced chronic exposure to toluene plus abstinence ("CTA") showed enhanced performance in a strategy set-shifting task using a between-session, but not a within-session test design. CTA also blunted operant and classical conditioning without affecting responding during a progressive ratio task. While CTA rats displayed normal latent inhibition, previous exposure to a non-reinforced cue enhanced extinction of classically conditioned approach behavior of these animals compared to air controls. To determine whether CTA alters the structural plasticity of brain areas involved in set-shifting and appetitive behaviors, we quantified basal dendritic spine morphology in DiI-labeled pyramidal neurons in layer 5 of the medial prefrontal cortex (mPFC) and medium spiny neurons in the nucleus accumbens (NAc). There were no changes in dendritic spine density or subtype in the mPFC of CTA rats while NAc spine density was significantly increased due to an enhanced prevalence of long-thin spines. Together, these findings suggest that the persistent effects of CTA on cognition are related to learning and memory consolidation/recall, but not mPFC-dependent behavioral flexibility.

1. Introduction

Toluene abuse typically consists of inhaling a concentrated vapor over a short period of time in order to achieve a hedonic, intoxicated state similar to other addictive substances (Howard, Bowen, Garland, Perron, & Vaughn, 2011). Like other drugs of abuse, this "high" is likely due to modulation of dopamine release in the striatum, potentially via enhancing excitatory signaling on mesolimbic dopamine neurons (Beckley, Evins, Fedarovich, Gilstrap, & Woodward, 2013; Lubman, Yücel, & Lawrence, 2008). In preclinical rodent studies, acute administration of abuse-level concentrations of toluene is an anxiolytic, has anti-depressant-like properties, produces a conditioned place preference, and impairs learning and motor coordination (Batis, Hannigan, & Bowen, 2010; Bowen, Wiley, & Balster, 1996; Gerasimov et al., 2003; Lopez-Rubalcava, Hen, & Cruz, 2000). These data parallel the behavioral profile of acute exposure to other addictive substances.

Since drug addiction may develop following chronic use, it is important to understand the effects of repeated, long-term toluene exposure on behavior and cognition. Results from preclinical studies reveal that chronic exposure to toluene can cause a wide range of cognitive and behavioral impairments including sensitization to drug-induced hyperlocomotion, impaired novel object recognition, spatial learning, and inhibitory avoidance (Batis et al., 2010; Baydas, Ozveren, Akdemir, Tuzcu, & Yasar, 2005; Huerta-Rivas et al., 2012). In addition, clinical studies in humans also report

Abbreviations: CTA, Chronic toluene exposure followed by protracted abstinence; PE, cue pre exposed; NPE, non cue pre exposed; mPFC, medial prefrontal cortex; NAc, nucleus accumbens core.

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cognitive deficits such as decreases in IQ and impairments in executive functions such response inhibition, behavioral flexibility, working memory, and attention (Howard, Balster, Cottler, Wu, & Vaughn, 2008; Lubman et al., 2008; Yuncu et al., 2015). Although these studies of chronic toluene exposure are essential to understanding the drugs' effects on cognition, most of them assessed behavioral performance shortly following the last toluene exposure. While important, it is also critical to examine whether there are changes in cognitive function following a more protracted period of abstinence.

Drug abstinence results in a negative emotional state, increased anxiety, and social withdrawal – all of which increase an individual's risk of relapse (Goodwin et al., 2002; McGregor, Callaghan, & Hunt, 2008; Wise & Koob, 2014). Understanding the behavioral profile during drug abstinence is essential for effective treatment of substance use disorders. The few studies concerning toluene's effects following protracted abstinence are somewhat inconsistent and results vary based on the cognitive measure tested. For example, while deficits in object recognition, operant conditioning, delay discounting, progressive ratio responding, and contingency monitoring have been observed, protracted abstinence from chronic toluene exposure does not affect Pavlovian-to-instrumental transfer, outcome devaluation, anxiety or spatial memory (Dick, Axelsson, Lawrence, & Duncan, 2014; Furlong et al., 2016; Lin, Ou, Chung, Pang, & Chen, 2010). Further, while inhalant-induced deficits in behavioral flexibility have been detected in humans after a short abstinence period (5–9 days), their effects in a protracted abstinence rodent model are subtle (Dick et al., 2014; Furlong et al., 2016; Yuncu et al., 2015).

One of the more commonly studied forms of behavioral flexibility involves training a subject to respond to a certain set of rules for a reward, and measuring the ability to adjust behavior when a new rule is introduced unexpectedly. Efficient completion of these tasks is critically-dependent on the integrity of the prefrontal cortex (Hamilton & Brigman, 2015). Moreover, disrupting communication between the medial prefrontal cortex (mPFC) and nucleus accumbens core (NAc) impairs shifting between strategies by increasing perseverative responding (Block, Dhanji, Thompson-Tardif, & Floresco, 2007). This circuitry is part of a larger network that controls the transition to habitual drug use, where prelimbic mPFC-NAc connectivity is essential for the initiation of drug-seeking behaviors (Everitt & Robbins, 2005; Stefanik et al., 2013). Both behavioral flexibility and drug addiction require structural modifications in the mPFC and NAc to permit the formation and maintenance of new synapses. The postsynaptic dendritic spine is a key component of this neuroplasticity, with long-thin immature spines giving way to mushroom-headed spines over the course of excitatory synaptic growth (Holtmaat, Wilbrecht, Knott, Welker, & Svoboda, 2006). While nearly every drug of abuse examined to date alters dendritic spine morphology in the mPFC and NAc (Mulholland, Chandler, & Kalivas, 2016; Spiga, Mulas, Piras, & Diana, 2014), it is not known whether similar changes occur following toluene exposure.

There is a particularly high incidence of inhalant abuse in adolescents due to the low cost and high availability of toluene-containing products (e.g. paint thinners, nail polish, permanent markers) (Johnston, O'Malley, Miech, Bachman, & Schulenberg, 2015). In the present study, adolescent rats were chronically exposed to abuse levels of toluene vapor and then allowed to recover in their home cage for a protracted abstinence (CTA). When rats reached adulthood we assessed two types of behavioral flexibility – strategy set-shifting and reversal learning – and examined the density and sub-types of dendritic spines in mPFC and NAc. The results from these studies show that toluene exposure during adolescence produces selective impairments in cognitive function during adulthood that are accompanied by alterations in dendritic spine morphology that are region- and spine-subtype specific.

2. Materials and methods

2.1. Animals

Sixty-seven male Sprague-Dawley Rats (post-natal day (P) 32 on arrival; Harlan Laboratories, Indianapolis, IN) were housed in pairs in polypropylene cages on a reverse light cycle (lights off at 0900 h) in a climate controlled room with *ad libitum* access to food and water unless otherwise noted. Rat identification numbers were written on the base of each tail using a permanent marker. Each rat was acclimated to handling for 5 min per day for at least 2 days prior to toluene exposure. All procedures were performed in compliance with the Medical University of South Carolina IACUC protocols.

2.2. Toluene exposure

On the day before the first toluene exposure, adolescent rats (P38) were habituated to the exposure chamber ($30 \times 30 \times 30$ cm) for 15 min. On each of the following 5 days (P39-43), a binge-like regimen was used to mimic adolescent human toluene abuse. Sessions consisted of two, 15 min exposures to 5700 ppm toluene generated using a sevoflurane vaporizer (Penlon Limited; flow rate 4 L/min, 8% volume). Each exposure was separated by 2 h of recovery in the home cage. We have previously used gas chromatography to validate this protocol for generating abuse-level toluene concentrations (Beckley et al., 2013). Importantly, these exposures fall within human consumption patterns: 15 min to several hours at 5000–15,000 ppm (Brouette & Anton, 2001; Bukowski, 2001). Interestingly, Gmaz, Yang, Ahrari, and McKay (2012) exposed Long-Evans rats to 5000 ppm toluene for 30 min and determined that the resulting brain toluene concentrations (500–1000 μmol/l) would be similar to those experienced by humans inhaling toluene-containing products. Similar exposure protocols have been used to study the effect of chronic exposure to abuse levels of toluene vapor (Bowen, Hannigan, & Cooper, 2009; Dick et al., 2014; Furlong et al., 2016; Moser & Balster, 1981). Control rats were exposed to chambers filled with air on the same schedule as above. Housing pairs were placed in the same drug treatment group to avoid potential exposure of air-treated controls to toluene. Animal weights were recorded every day following toluene exposure, and once every 3–5 days thereafter.

2.3. Behavioral flexibility

2.3.1. Lever press training

Rats (eighteen toluene-, seventeen air-treated) were first habituated to 20% sweetened condensed milk (SCM), the reward used throughout these studies. During reward exposure, each rat pair was given free access to 10 ml SCM for two days before exposure to the operant chambers. Subjects were monitored to ensure both rats sampled the SCM. Lever press training was subsequently conducted in operant chambers (Med Associates, St. Albans, VT) that began during adulthood (P60) and proceeded as described previously (Brady & Floresco, 2015). Briefly, rats were first trained to lever press on a fixed-ratio (FR) 1 schedule for 45 μl SCM dispensed from a central feeding well over the course of three phases (1 −3). Phase 1 (30 min session) began with both levers extending, each of which were reinforced on an FR1 schedule. Rodents moved on to phase 2 if they made 50 responses for two consecutive days. Phase 2 was identical to phase 1, except that levers retracted 20 s when pressed and then were presented again. Rodents progressed to