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Repeated toluene exposure increases the excitability of layer 5 pyramidal neurons in the prefrontal cortex of adolescent rats

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ABSTRACT

Despite serious health effects, volatile industrial products containing toluene are deliberately inhaled for their psychoactive actions, mainly among adolescents and young adults. Chronic toluene inhalation induces multiple alterations at the cellular and behavioral level; however, modifications of neuronal networks associated with the reward system after repeated toluene exposure are not thoroughly characterized. Here we used whole-cell recordings to determine the effects of repeated exposure to toluene (1000, 4000 or 8000 ppm for 30 min, twice a day, for ten days) on the neurophysiological properties of prelimbic layer 5 pyramidal neurons of the medial prefrontal cortex (mPFC) in adolescent male Wistar rats. Neurons from animals repeatedly exposed to toluene showed a concentration-dependent increase in action potential firing discharge. This increase was related to a reduction of the small-conductance calcium-activated potassium current (after-hyperpolarization current, I_{AHP}) that controls the firing frequency of neurons. Likewise, toluene altered the kinetics of the action potential. The hyperexcitability seen in toluene-exposed animals was also associated with an increase in the glutamatergic spontaneous synaptic activity converging on mPFC neurons. In summary, repeated toluene exposure enhances the excitability of prelimbic layer 5 pyramidal neurons of the mPFC in adolescent rats.

1. Introduction

Inhalant misuse, also referred to as inhalant abuse or volatile substance misuse, is the intentional inhalation of volatile substances to induce mind-altering effects (Balster et al., 2009; Rees et al., 1985). Inhalants are present in legal, inexpensive and readily accessible commercial products such as sprays, solvents, paint thinners, fuels and glues (Balster et al., 2009). In the inhalant abuse setting users are exposed to organic solvents at concentrations that are several thousand-fold higher (up to 15,000 ppm) than those found in the occupational setting (Bowen et al., 2006; Bukowski, 2001; Hathaway et al., 2004; Marjot and McLeod, 1989). Inhalant misuse is a public health problem that causes significant worldwide morbidity and mortality due to long-term toxicity, attention and memory impairment, sleep disturbances and other disorders (Real et al., 2015; Ridenour et al., 2007; Yücel et al., 2008).

The most commonly inhaled substance among solvent users is toluene. The acute effects of toluene are similar to those produced by several central nervous system (CNS) depressant drugs (Balster, 1998). Toluene exposure in episodes that mimic binge patterns of intoxication (1000–8000 ppm, for 15–30 min) produces antianxiety-like effects (Beasley et al., 2010; López-Rubalcava et al., 2000) decreases motor coordination (Bowen et al., 1996) and impairs cognition (Bale et al., 2005; Baydas et al., 2003; Huerta-Rivas et al., 2012; Win-Shwe et al., 2012).

Inhalant misuse is particularly relevant among adolescents and young adults (Hynes-Dowell et al., 2011; Johnston et al., 2016; Medina-Mora and Real, 2008; Villatoro et al., 2011). This problem is worsened because adolescence represents the final transitional period of behavioral and cognitive maturation. During this stage, the brain undergoes multiple adjustments, including neuronal pruning, neurotransmission refinement and regional brain maturation (Crews and Boettiger, 2009; Spear, 2000). The prefrontal cortex (PFC) is one of the brain areas that exhibit more signs of maturation during adolescence (Blakemore, 2008; Casey et al., 2008; Sturman and Moghaddam, 2011). This region integrates stimuli from multiple cortical and subcortical structures and functions, such as planning processing, working memory, impulse control, and risk assessment. The prelimbic region of mPFC is involved in learning and decision making and modulates the activity of the nucleus accumbens, a subcortical structure critically involved in the

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reward circuit (Euston et al., 2012; Rushworth et al., 2011).

The negative impact of substance abuse on the PFC has been widely documented for ethanol (Ward et al., 2014), but studies using toluene are scarce despite similarities in action mechanisms between these drugs. Both ethanol and toluene increase cortical dopamine release (Riegel et al., 2007; Williams et al., 2005), are non-competitive NMDA receptor antagonists (Cruz et al., 1998) and exert positive allosteric $GABA_A$ receptor modulation (Beckstead et al., 2000). In addition, toluene modulates a wide range of ligand-operated neurotransmitter receptors and voltage-sensitive ion channels, which include, but are not limited to sodium, potassium and calcium channels (reviewed in Beckley and Woodward, 2013; Cruz et al., 2014).

Perfusion of toluene (1 to 3 mM) on acute mPFC slices does not modify passive properties or the intrinsic excitability of mPFC neurons from adolescent rats (Beckley and Woodward, 2011). On the other hand, a recent study found that brief toluene inhalation (10,500 ppm) transiently alters, in a regional-specific manner, mPFC excitability (Wayman and Woodward, 2017). The same group of researchers found that repeated (6) pairings of 3000 ppm toluene exposure that induced a conditioned place preference: a) increased the evoked firing of infralimbic mPFC pyramidal neurons that project to the nucleus accumbens (NAc) core; b) decreased evoked firing of infralimbic pyramidal neurons projecting to the NAc shell, and c) had no effect on the excitability of prelimbic neurons regardless of the NAc projection target of the recorded neuron (Wayman and Woodward, 2018). Here, we investigate the effects of a sub-chronic exposure to toluene on the intrinsic excitability of cortical neurons of the prelimbic mPFC in adolescent rats. Our data provide evidence that toluene increases the excitability of prelimbic mPFC pyramidal neurons in a concentration-dependent manner by reducing the amplitude and duration of the slow after-hyperpolarization potential and increasing spontaneous synaptic activity.

2. Materials and methods

2.1. Animals

A total of 50 adolescent male Wistar rats (postnatal day (PN) 25–37) were used in this study. Animals were bred and housed under a 12:12 h light/dark cycle in a climate-controlled room at 22 ± 2 °C at our local animal facility with ad libitum access to food and water under continuous veterinary supervision. The experimental procedures were carried out in strict accordance with the Mexican Official Norm for utilization and care of laboratory animals "NOM-062-ZOO-1999" and complied with the local Ethics Committee of our Institution (authorization numbers 0101-14 and 0090-14), the National Institutes of Health guidelines (NIH, 2011) and ARRIVE guidelines (Kilkenny et al., 2013) for animal research.

2.2. Drugs

Toluene (99.8% HPLC grade), 1(*S*),9(*R*)-(−)-bicuculline methiodide (3-Triphenyl methylaminomethyl) pyridine (UCL-2077) and 4-hydroxyquinoline-2-carboxylic acid (kynurenic acid) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA). All drugs except toluene were superfused at a rate of 3–5 ml/min.

2.3. Toluene exposure

Independent groups of animals were exposed to air, 1000, 4000 or 8000 ppm toluene for 30 min, twice a day, 6 h apart, for five consecutive days. Animals were left untreated for the weekend and then re-exposed to air or toluene twice a day for another five days in a static exposure chamber as previously described (Rivera-García et al., 2015). Briefly, each animal was placed into a 27-l cylindrical jar that had a rubber gasket fixed to the rim of the jar to ensure a close-fitting seal. The jar was then hermetically closed with a Plexiglas cover that had injection ports and a fan projecting into the chamber. The liquid solvent was delivered with a syringe through an injection port onto a piece of paper suspended below the fan, which was then turned on for rapid solvent evaporation and dispersion. We used the following equation for calculating the amount of solvent to be injected into the chamber to achieve specific concentrations (Nelson, 1971): $Vl = ((MW\ Cppm\ Vs)/\rho)\ ((P(10^{-6}) / RT)$ where: Vl = volume of the solvent injected into the closed system to achieve the desired concentration in ppm (Cppm); MW = molecular weight (toluene = 92.14 g mol^{-1}); Vs = volume of the closed-loop system (exposure chamber: 27 l); ρ = density (toluene = 0.865 g ml^{-1}), P = pressure (0.77 atm in Mexico City), R = ideal gas constant (0.082 l atm mol^{-1} K^{-1}), T = 293 K. Accordingly, the amounts of toluene injected into the chamber were 92 µl for 1000 ppm, 368 µl for 4000 ppm and 736 µl for 8000 ppm. Nominal concentrations were confirmed using a photoionization detector (PhoCheck Tiger, Ion Science, LTD, Cambs, UK). The actual concentrations varied from the 1st and the 30th minute after injection as follows: for a theoretical 1000 ppm concentration = from 1221 to 922 ppm; for 4000 ppm = from 4559 to 3580; for 8000 ppm = from 8802 to 7468 (Fig. 1, Supplementary figure). Toluene concentrations were chosen based on previous reports in animals (Beyer et al., 2001; Callan et al., 2017; Rivera-García et al., 2015) and relevance to solvent intoxication (Bowen et al., 2006; Bukowski, 2001; Hathaway and Proctor, 2004).

2.4. Brain slice preparation

Rats were deeply anesthetized with sodium pentobarbital (50 mg/kg i.p.) and decapitated 18 h after the 20th solvent exposure. The brains were quickly removed and placed in frozen sucrose solution containing (in mM): 210 sucrose, 25 $NaHCO_3$, 10 glucose, 2.8 KCl, 2 $MgSO_4$, 1.25 NaH_2PO_4, 4 $MgCl_2$ and 1 $CaCl_2$, bubbled with 95%:5% O_2: CO_2. The modification in the Ca^{2+}/Mg^{2+} ratio and the substitution of sucrose for D-glucose were used to minimize the neuronal damage following the mechanical slicing. The medial PFC (mPFC) was dissected out and cut into 385 µm coronal slices using a vibratome (Leica VT1000 S, Nussloch, Germany). Slices were incubated for 30 min at 35 °C and then stabilized for no < 1 h at room temperature in an incubation solution of the following composition (in mM): 125 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 25 $NaHCO_3$, 10 glucose, 4 $MgCl_2$ and 1 CaCl; bubbled with 95%:5% CO_2:O_2. Slices were individually placed in the recording chamber and superfused at a rate of 3–5 ml/min with artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 3.0 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 1 $CaCl_2$, 2 $MgCl_2$, and 10 D-glucose. The time elapsed between the last toluene exposure and the beginning of the experiment was 20 ± 1 h, which allowed for drug elimination.

2.5. Whole cell recordings

Layer 5 pyramidal cells located in the prelimbic sub-region of mPFC were visually identified using an upright infrared-differential interference contrast (IR-DIC) microscope (Nikon Eclipse FN1, Nikon Instruments, USA). Recordings were obtained using an Axopatch-1D amplifier (Molecular Devices, USA). The analog signals acquired during our experiments were real-time filtered via a Humbug noise eliminator and additional P-Clamp digital filters (8-pole Bessel with cut-off 5) were used at convenience for each analyzed cell in order to increase the signal-to-noise ratio. Borosilicate glass electrodes (4–8 MΩ resistance) were filled with standard solution containing (in mM): 120 K-$MeSO_4$, 10 NaCl, 10 KCl, 0.5 EGTA, 4 ATP-Mg, 0.3 GTP, 10 HEPES and 14 phosphocreatine; pH 7.2–7.35 and osmolarity 280–300 mOsm. In experiments intended to isolate the afterhyperpolarization current (I_{AHP}) in voltage clamp mode, the EGTA concentration was reduced to 0.2 mM to favor amplitude and total area of the isolated current. The access resistance (≤ 25 MΩ) was monitored during the length of the experiments to ensure high-quality recordings and experiments were discarded if the resistance changed > 20% throughout recordings. For