Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

NMDA receptor binding is reduced within mesocorticolimbic regions following chronic inhalation of toluene in adolescent rats

Alec Lindsay Ward Dick[a,b], *Tine Pooters*[a], *Sarah Gibbs*[a], *Emma Giles*[a], *Ashleigh Qama*[a], *Andrew John Lawrence*[a], *Jhodie Rubina Duncan*[a,c,*]

[a]*Division of Behavioural Neuroscience, Florey Institute of Neuroscience and Mental Health, University of Melbourne, Melbourne, Victoria 3010, Australia*

[b]*Department of Stress Neurobiology and Neurogenetics, Max Planck Institute of Psychiatry, 80804 Munich, Germany*

[c]*Department Anatomy & Neuroscience, University of Melbourne, Melbourne, Victoria 3010, Australia*

ARTICLE INFO

Article history:
Accepted 23 July 2015
Available online 30 July 2015

Keywords:
Inhalant abuse
Animal model
Autoradiography
qPCR
NMDA
GluN2B

ABSTRACT

The purposeful inhalation of volatile solvents, such as toluene, to induce self-intoxication is prevalent, particularly within adolescent populations. Chronic misuse results in cognitive and neurobiological impairments, as well as an increased risk for addictive behaviours in adulthood. Toluene-induced neuroadaptations within mesocorticolimbic circuitry are thought, in part, to mediate some of the adverse outcomes of toluene misuse, however our understanding of the neuroadaptive processes remains equivocal. An understanding of these processes is particularly important relative to exposure that occurs during adolescence and at concentrations that reflect various patterns of use. Therefore, we exposed male adolescent Wistar rats (postnatal day [PN] 27) to either air or low or high concentrations of inhaled toluene in a chronic and intermittent fashion (CIT, 3,000 or 10,000 ppm) for 1 h/day, 3–5 times per week for 4 weeks to model different patterns of human inhalant abuse. Brains were subsequently analysed using autoradiography, qPCR and immunohistochemistry 3 days following the exposure period to investigate toluene-induced neuroadaptations within mesocorticolimbic circuitry. In CIT-exposed rats binding to N-methyl-D-aspartate (NMDA) receptors containing the GluN2B subunit, as determined using [^{3}H]-ifenprodil, was decreased in a concentration-related manner in the caudal cingulate cortex, dorsal striatum and accumbens; however, this was not associated with changes in GluN2B protein expression. There were no differences in [^{3}H]-epibatidine binding to heteromeric neuronal nicotinic acetylcholine (nACh) receptors. Relative expression of mRNA transcripts encoding NMDA, nACh, γ-aminobutyric acid type-A ($GABA_A$) and dopamine receptor subunits was unchanged in all regions assessed following CIT. Our data suggest that adolescent CIT exposure impacts NMDA receptors within regions of corticostriatal circuitry, possibly via post-translational mechanisms. Dysfunctional glutamatergic signalling within corticostriatal regions may contribute to the adverse outcomes observed following adolescent toluene abuse.

*Corresponding author at: Florey Institute of Neuroscience and Mental Health, L3, Kenneth Myer Building, Cnr Genetics Lane on Royal Parade, University of Melbourne, Parkville, Victoria 3010, Australia. Fax: +613 09353107.
E-mail address: Jhodie.duncan@florey.edu.au (J.R. Duncan).

http://dx.doi.org/10.1016/j.brainres.2015.07.037
0006-8993/

1. Introduction

Intentional inhalation of volatile organic solvents, such as toluene, is a major health concern worldwide, especially within adolescent populations (Lubman et al., 2006). Abuse during this time is a predictor of substance abuse disorders later in life and has a significant co-morbidity with neuropsychiatric disorders (Wu et al., 2004). Inhalant abuse is also associated with long-term adverse outcomes including behavioural and cognitive dysfunction (Yucel et al., 2008; Dick et al., 2014). Such outcomes may be due to toluene-induced neuroadaptations within discrete neural circuitry following repeated exposure (Beckley et al., 2013a).

Toluene is found in many household products that are commonly abused, such as paints, glues and aerosols. It has high potential for abuse as it can elicit a conditioned place preference (CPP) in rodents (Funada et al., 2002) and is self-administered in mice (Blokhina et al., 2004) and non-human primates (Weiss et al., 1979). Similar to other drugs of abuse, toluene's acute hedonic properties are thought to be mediated, at least in part, via regulation of signalling within mesocorticolimbic circuitry (Riegel and French, 1999; Riegel et al., 2007). Toluene's effects on behaviour may relate to its ability to regulate both inhibitory and excitatory neurotransmission, and receptor subunit expression, within discrete regions of this circuitry. Notably, exposure to toluene results in consistent regulation of N-methyl-D-aspartate (NMDA) receptors in particular (Cruz et al., 1998; Williams et al., 2005; MacIver, 2009; Beckley and Woodward, 2011).

NMDA receptors are tetrameric complexes consisting of two GluN1 and two GluN2 subunits. The GluN2A and GluN2B subunits predominate, particularly within forebrain structures, and show differential channel kinetics (Watanabe et al., 1993; Monyer et al., 1994; El Gaamouch et al., 2012). Toluene rapidly and reversibly inhibits NMDA receptors *in vitro* with the GluN1/GluN2B conformation reportedly the most sensitive to toluene-induced inhibition (Cruz et al., 1998). Moreover, toluene exposure in adult rodents can differentially alter GluN2B protein expression in discrete brain regions (Williams et al., 2005) as well as γ-aminobutyric acid type-A ($GABA_A$) receptor subunits. In contrast, neonatal toluene administration (500 mg/kg, i.p., postnatal day [PN] 4–9) in rats results in increased GluN2A expression within the dorsal hippocampus (dHPC) and cerebellum at the onset of adolescence (PN 30), with alterations of GluN2B only observed within the cerebellum (Lee et al., 2005). In a validated rodent model we have previously observed discrete cognitive deficits following chronic intermittent toluene (CIT) exposure during adolescence in rats. These deficits were present within the first week after exposure to toluene with deficits lasting at least 10 weeks suggesting long-term neuroadaptive responses within the mesocorticolimbic circuitry (Dick et al., 2014). Furthermore, changes in cognitive processes parallel with toluene-induced glutamatergic dysfunction mediated by NMDA receptors in our model (Duncan et al., 2014).

Cholinergic signalling is also affected following exposure to toluene with reduced extracellular acetylcholine release observed within the striatum and hippocampus (HPC) *in vivo* (Stengard, 1994; Honma and Suda, 2004). Neonatal (PN 4–9) and pre/early adolescent (PN 25–30) toluene administration (500 mg/kg, i.p.) in rats also alters the behavioural sensitivity to acute nicotine administration in early adulthood in the absence of alterations neuronal nicotinic acetylcholine (nACh) receptor subunit expression (Chan et al., 2008). Toluene inhibits neuronal nACh receptors *in vitro*, with the $\alpha_4\beta_2$ subtype being most sensitive (Bale et al., 2002; Bale et al., 2005). Interestingly, $\alpha_4\beta_2$ nACh receptors are inhibited by toluene at similar concentrations as GluN1/GluN2B NMDA receptors emphasising a comparative sensitivity of these two receptor subtypes to toluene-induced regulation *in vitro* (Cruz et al., 1998; Bale et al., 2002; Bale et al., 2005). Thus, the sensitivity of $\alpha_4\beta_2$ nACh receptors to toluene may implicate a further neurotransmitter system affected via chronic exposure, although this remains to be elucidated especially following adolescent exposure.

Recent evidence suggests that both glutamatergic and cholinergic synapses are labile throughout adolescence, particularly within the medial prefrontal cortex (mPFC) (Counotte et al., 2012; Wang et al., 2013; Flores-Barrera et al., 2014). Thus, these systems may be vulnerable to CIT-induced alteration during this period. Moreover, toluene's neurochemical and neuroadaptive properties appear to be concentration-dependent (Gerasimov et al., 2002; Koga et al., 2007; Beckley et al., 2013a) such that high (>5000 ppm) as opposed to moderate (~3000 ppm) concentrations of toluene have differential pharmacological effects. Frequency of exposures must also be considered as initial experimentation with inhalants in humans often progresses from frequent inhalation of moderate concentrations via "sniffing," to less frequent episodes of extended inhalation of higher vapour concentrations via "huffing" or "bagging" as inhalant misuse escalates (Henretig, 1996; Kurtzman et al., 2001). Together, concentration and frequency of toluene exposure mediate diverse modifications including behavioural outcomes (Beckley et al., 2013a; Batis et al., 2010).

As we have previously observed both cognitive and behavioural deficits following chronic intermittent exposure to toluene during adolescence (Dick et al., 2014), which paralleled with long-term changes in glutamatergic signalling in our model (Duncan et al., 2014), this study examined neurochemical changes within discrete regions of the mesocorticolimbic system 3 days after the last exposure to toluene to investigate potential neurobiological mechanisms subserving these changes. We hypothesised that NMDA receptors would be susceptible to toluene-induced adaptations following adolescent CIT exposure. Based on observations from human settings (Henretig, 1996), we also sought to examine the effects of concentration and/or frequency upon these neurobiological parameters. We hypothesised that models reflecting early (moderate toluene concentration, 3000 ppm, at high frequency, 5 days/week) compared to later (high toluene concentration, 10,000 ppm, less frequently, 3 days/week) stages of inhalant abuse would be less likely to induce neurochemical adaptations. We observed a concentration-dependent decrease in receptor binding specifically to NMDA receptors containing the GluN2B subunit in the caudal anterior cingulate cortex (ACg), dorsal striatum (DS) and nucleus accumbens (NAc); however this was not associated with changes in genes encoding NMDA receptor subunits or GluN2B protein expression. Moreover, no changes to nACh receptor binding, or genes encoding nACh, $GABA_A$ or