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Binge Toluene Exposure Alters Glutamate, Glutamine and GABA in the Adolescent Rat Brain as Measured by Proton Magnetic Resonance Spectroscopy*

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Abstract

Despite the high incidence of toluene abuse in adolescents, little is known regarding the effect of binge exposure on neurochemical profiles during this developmental stage. In the current study, the effects of binge toluene exposure during adolescence on neurotransmitter levels were determined using high-resolution proton magnetic resonance spectroscopy *ex vivo* at 11.7 T. Adolescent male Sprague-Dawley rats were exposed to toluene (0, 8,000 , or 12,000 ppm) for 15 min twice daily from postnatal day 28 (P28) through P34 and then euthanized either one or seven days later (on P35 or P42) to assess glutamate, glutamine, and GABA levels in intact tissue punches from the medial prefrontal cortex (mPFC), anterior striatum and hippocampus. In the mPFC, toluene reduced glutamate one day after exposure, with no effect on GABA, while after seven days, glutamate was no longer affected but there was an increase in GABA levels. In the hippocampus, neither GABA nor glutamate was altered one day after exposure, whereas seven days after exposure, increases were observed in GABA and glutamate. Striatal glutamate and GABA levels measured after either one or seven days were not altered after toluene exposure. These findings show that one week of binge toluene inhalation selectively alters these neurotransmitters in the mPFC and hippocampus in adolescent rats, and that some of these effects endure at least one week after the exposure. The results suggest that age-dependent, differential neurochemical responses to toluene may contribute to the unique behavioral patterns associated with drug abuse among older children and young teens.

*Supplementary data from this study are provided with the online version of this article. See Appendix A.

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Keywords

Toluene; adolescents; glutamate; GABA; magnetic resonance spectroscopy; neuropharmacology; imaging

1. Introduction

Toluene (methylbenzene) is an aromatic hydrocarbon used as an industrial organic solvent in the production of spray paints, paint thinners, and gasoline (Lubman et al., 2008) that is misused globally by adolescents for its euphoric effects (NIDA, 2005). While adolescents are much more likely than adults to abuse toluene (SAMHSA, 2008), research in rodents has typically focused on adult animals (Flanagan and Ives 1994; Brouette and Anton 2001; Bowen et al., 2006; Cruz and Bowen, 2008).

Inhalant abuse is characterized by variable patterns of binge, high-concentration exposures that are brief and repeated (Watson, 1982). Inhalations consist of deep breaths in short bouts up to 15 or 30 minutes, or breathing vapors over many hours, or repeating the short bouts throughout a day and/or across days (Brouette and Anton, 2001; Bukowski, 2001; Oliver and Watson, 1977; Watson, 1977, 1980). We previously developed and validated an animal model that mimics the binge patterns and high concentrations of exposure seen in toluene abuse (Bowen et al., 2005; Bowen et al., 2007a; Bowen et al., 2007b; O'Leary-Moore et al., 2007).

The mechanism(s) of action for the psychoactive and neurotoxic effects of toluene require clarification. Animal studies using a variety of routes of administration show toluene modulates a number of neurotransmitter systems, similar to those of other drugs of abuse [see reviews (Balster, 1998; Bowen et al., 2006)]. Toluene exposure induces neuroplastic changes in GABA and glutamate receptors, although the mechanism of these effects remain to be determined (Bale et al., 2005b; Cruz et al., 2000; Maciver, 2009; Williams et al., 2005). Previous research from our group using proton magnetic resonance spectroscopy (¹H-MRS) *ex vivo*, revealed that acute toluene exposure via inhalation reduced hippocampal GABA levels in juvenile (O'Leary-Moore et al., 2007) and adolescent rats, but not in adult rats (O'Leary-Moore et al., 2009). Toluene also reduced glutamate levels in the hippocampus of juveniles (i.e., postnatal day 21 – P21) (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). While these studies indicate age-dependent neurochemical effects of toluene, the neurobiological consequences of patterns of toluene exposure characteristic of abuse, particularly in adolescence, require further investigation.

The present study was designed to expand on our previous research with acute toluene exposures by examining the effects of repeated (several days), brief (15-min) high-concentration (8,000 ppm & 12,000 ppm) toluene inhalation on GABA, glutamine and glutamate levels in the adolescent rat brain. These behaviorally relevant concentrations were used in our previous studies of acute toluene effects on both behavior (Batis et al., 2010; Bowen et al., 2005; Bowen et al., 2009a; Bowen et al., 2009b) and high-resolution ¹H-MRS-visible neurochemical profiles (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). Further, neurochemical profiles were assessed via ¹H-MRS 1 or 7 days after cessation of toluene exposure to examine the persistence and/or delay of effects. The effects of toluene on GABA, glutamine and glutamate levels were determined in intact tissue samples from medial prefrontal cortex (mPFC), anterior striatum and hippocampus. These areas were selected because toluene has been shown to affect these areas in clinical studies (Aydin et al., 2002; Aydin et al., 2003; Deleu and Hanssens, 2000; Kamran and Bakshi, 1998; Yamanouchi et al., 1995), and/or in prior animal studies (e.g., (Ameno et al., 1992;

Gerasimov et al., 2002)). We hypothesized that repeated binge toluene exposure would reduce cortical, hippocampal, and striatal glutamate, glutamine, and GABA levels, and that glutamate, glutamine, and GABA levels would recover after 7 days of abstinence.

2. Methods

All procedures were approved by the Wayne State University Institutional Animal Care and Use Committee and all animal care and use were in accordance with all applicable portions of the Animal Welfare Act and the current NIH "Guide for the Care and Use of Laboratory Animals". The University maintains Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited animal facilities under the Division of Laboratory Animal Resources.

2.1 Experimental Design and Toluene Exposure

Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI) arrived on postnatal day 24 (P24) and acclimated for 4 days prior to experimentation. Except for the period of toluene exposure, animals were housed in a temperature (~24°C) and humidity controlled (35%-40%) environment with a 12 hour light/dark cycle (lights on at 0700). For one week, from P28 through P34, rats were exposed each day to one of three concentrations of toluene (0 ppm, 8,000 ppm, or 12,000 ppm; Fisher Scientific Co., Fairlawn, NJ) for 15 min twice daily with 2 hours between exposures. Details of the exposure regime and the rationale (outlined above) for the timing, concentration and duration of exposure used are the same as in our prior research (Bowen et al., 2005; Bowen et al., 2007a; Bowen et al., 2007b; O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). Briefly, vapor exposures were given in 36-liter cylindrical glass chambers with Plexiglas® lids in a chemical fume hood. Rats were placed one at a time for 15 min into the sealed chambers and toluene (0.0 ml for 0 ppm air controls, 1.36 ml for 8,000 ppm, 2.05 ml for 12,000 ppm) was injected through a port into a diffuser which volatilized the toluene and spread it throughout the chamber. Chambers were evacuated between animals. Single wavelength monitoring infrared spectroscopy (Miran 1-A, Foxboro, Analytical, North Haven, CT) was used periodically to ensure consistent ambient toluene concentrations and proper chamber clearance. Actual toluene concentrations in inhalation chambers during the 8,000-ppm and 12,000-ppm exposure conditions were within 2% of nominal within 1 to 2 minutes and did not deviate significantly from that asymptote during an entire 15-min session.

2.2 Tissue Collection

One day after the last toluene exposure (P35) half of each exposure level group was euthanized. The remaining animals were left undisturbed in their home cages until they were euthanized 7 days later on P42. Final sample sizes (N) were 6 – 8 per toluene exposure level group.

Animals were euthanized by decapitation in the absence of anesthesia. Brains were removed rapidly, placed into an ice-cold brain matrix, and then sliced into 2-mm thick coronal slices. Coronal slices were placed on a block of solid CO₂ and 2.1 mm diameter punches were taken from the mPFC, anterior striatum and hippocampus. Dissections were guided by landmarks, referring to atlas coordinates (Paxinos, 2007) and as specified in our previous studies (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). Tissue samples were stored at -80°C until ¹H-MRS measurement.

2.3 High Resolution Magic Angle Spinning ¹H-MRS Analysis Ex Vivo

Frozen intact tissue samples were weighed and placed into a 10-μl Bruker zirconium rotor containing buffer (pH = 7.4; 100 mM K₂HPO₄/KH₂PO₄, 200 mM HCOONa, 1 g/l NaN₃

diluted 50% with D₂O containing 3 mM trimethylsilyl-propionate [TSP; Sigma, St Louis, MO]), as detailed previously (O'Leary-Moore et al., 2007; Perrine et al., 2009). Briefly, the rotor containing the sample was placed into a Bruker spinning probe maintained at 4°C in a vertical wide-bore (8.9 cm) Bruker 11.7 T magnet (Bruker Biospin Corp., Billerica, MA), where the rotor was spun at 4.2 ± 0.002 kHz at 54.7° (i.e., the magic angle) relative to the static magnetic field B₀. After a pre-saturation pulse for water suppression, spectra were acquired with a 1-D Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence and data acquisition was performed using Bruker-XWINNMR version 3.6 software. Spectral data were analyzed by a custom LCModel version 6.1-4 (Provencher, 2001), which was established using a custom basis set of 27 brain metabolites and non-specific lipid signals with chemical shifts between 1.0 – 4.2 ppm. LCModel data were corrected for initial tissue weight and expressed as mmol metabolite per mg tissue. For ¹H-MRS measurements, samples of a given age and brain region were ran together (e.g. 0 ppm, 8,000 ppm, and 12,000 ppm samples from mPFC of P35 rats were measured on a given day), and within each run samples were randomized.

2.4 Statistics

Data were analyzed with univariate analyses of variance (ANOVA) within each brain region. Toluene treatment Group (3 exposure levels: 0 ppm, 8,000 ppm or 12,000 ppm), and Time (1 day or 7 days after exposure) were fixed, between-subjects factors. As noted above, glutamate (GLU), glutamine, and GABA levels were assessed because of *a priori* hypotheses regarding their alterations based on previous data (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). Supplementary data on other neurochemicals are provided in Appendix A. Dunnett *post hoc* tests were used to further assess significant ANOVA results. An alpha level of $p < 0.05$ was maintained throughout the analyses.

3. Results

Similar to our previous reports (Bowen et al., 2005), animals inhaling toluene in the present study displayed initial qualitative increases in activity followed by concentration-dependent decreases in activity (data not shown).

3.1 Glutamate (GLU)

Significant effects of Time on GLU were seen in all three brain regions: mPFC ($F(1,42) = 65.43$, $p < 0.001$; Figure 1A), anterior striatum ($F(1,37) = 19.94$, $p < 0.001$; Figure 2A), and hippocampus ($F(1,35) = 7.38$, $p < 0.01$; Figure 3A). *Post-hoc* analyses revealed that GLU levels were significantly lower 7 days after exposure in each region compared to 1 day post exposure. Significant toluene Group $\times$ Time interactions were also found for GLU in mPFC ($F(2,42) = 4.46$, $p < 0.01$; Figure 1A) and hippocampus ($F(2, 35) = 4.80$, $p < 0.05$; Figure 3A). Follow-up analyses revealed that repeated exposure to 8,000 ppm or 12,000 ppm toluene lowered GLU levels in mPFC 1 day after exposure ($F(2,21) = 5.38$, $p < 0.05$; Figure 1A), compared to 0 ppm controls, but those effects were no longer present 7 days after toluene exposure. In contrast, there were no significant differences in GLU levels in the hippocampus 1 day after toluene treatment, but 7 days later, the 12,000 ppm toluene exposed group had higher GLU levels in the hippocampus than the 0-ppm air-exposed control group ($F(2,19) = 4.93$, $p < 0.05$; Figure 3A).

3.2 Glutamine

Although toluene did not have a significant main effect on glutamine, significant effects and interactions involving the Time factor were seen. Glutamine levels were higher in the mPFC ($F(1,42) = 19.44$, $p < 0.001$; Figure 1B) and anterior striatum ($F(1,37) = 16.87$, $p < 0.001$; Figure 2B) 7 days after exposures compared to levels 1 day post-exposure. In contrast,

glutamine levels in the hippocampus were lower after 7 days than after 1 day ($F(1,35) = 16.51, p < 0.001$; Figures 3B). In addition, a significant toluene treatment Group $\times$ Time interaction was found for the hippocampus ($F(2,35) = 3.64, p < 0.05$; Figure 3B). Inspection of Figure 3B suggests that relative to 0 ppm control levels at each time point, glutamine levels tended to be reduced by toluene after 1 day but increased by toluene after 7 days. However, the larger effect seems to be a reduction in glutamine levels in the 0 ppm group between 1 and 7 days. Follow-up analyses failed to detect any statistically significant effects of toluene exposure at either time point.

3.3 GABA

There were no significant main effects of toluene treatment on GABA levels. However, there were significant effects of Time on GABA in the anterior striatum ($F(1,37) = 71.21, p < 0.001$; Figure 2C) and hippocampus ($F(1,35) = 31.57, p < 0.001$; Figure 3C). *Post-hoc* tests indicated that GABA levels were significantly lower after 7 days compared to levels measured 1 day after exposure ($p < 0.05$; Figures 2C and 3C). A significant toluene treatment Group $\times$ Time interaction for GABA was evident in the mPFC ($F(2,42) = 5.14, p < 0.01$; Figure 1C). Follow-up analyses revealed that although no significant differences were found in cortical GABA levels in animals 1 day after toluene exposure, rats that had been exposed to either 8,000 ppm or 12,000 ppm toluene had higher levels of GABA as compared to 0 ppm controls when assessed 7 days later ($F(2,21) = 4.41, p < 0.05$; Figure 1C).

4. Discussion

The current experiment assessed effects of repeated, brief (15-min), binge-pattern, high-concentration toluene exposure during adolescence on levels of glutamate, glutamine and GABA in rat brain, measured by high-resolution *ex vivo* ^1H -MRS. The concentrations (8000 ppm & 12,000 ppm) and binge pattern of toluene exposure were chosen to model characteristics of exposure that mimic human teen inhalation abuse. The main finding was that repeated binge pattern toluene inhalation produced brain region- and concentration-dependent effects in glutamate (GLU) and GABA, but not glutamine. In addition, comparisons of the effects of toluene 1 and 7 days after repeated inhalation exposure also revealed what may be time-dependent changes in neurochemical levels. These results extend our previous research which showed that acute, one-time binge toluene exposure differentially affected amino acid neurotransmitters, in concentration-, brain region-, and age-dependent manners (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). The observation that toluene effects are dependent on concentration, region, and abstinence period highlights neurobiological specificity and may provide insight into a particular vulnerability in the adolescent brain to toluene.

Contrary to our predictions, a repeated binge-like pattern of toluene inhalation mimicking clinical solvent abuse did not affect striatal GLU, glutamine, or GABA (Figure 2) in the adolescent rat. The absence of a repeated toluene effect on striatal glutamate is similar to our previous observations after acute binge inhalation for one day in juvenile, adolescent or adult rats (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009) suggesting that glutamate afferents to the anterior dorsal striatum from cortex are relatively resistant to repeated toluene exposure. A single exposure to toluene decreased GABA levels in the anterior striatum of both juvenile and adult rats – but not adolescent rats – when measured shortly after exposure (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). Our combined observations suggest an age-dependent effect of toluene exposure and show that the anterior striatum in the adolescent rat is less sensitive to changes in GABA following acute or repeated binge toluene exposure than are older and younger rats. In adult rats, 10 days of toluene exposure (8000 ppm; 30 min/day) increased levels of the α_1 subunit of striatal GABA_A receptors (Williams et al., 2005), an effect that may compensate for toluene-

induced decreases in striatal GABA. In both adult and young adults animals, toluene has shown anticonvulsant properties that are, in part, mediated by GABA (Chan et al., 2006) and conditioned place preference to toluene in adult rats is reduced by the GABA transaminase inhibitor vigabatrin (Lee et al., 2004). While more research with adolescents is necessary, the present data suggest that homeostatic control of striatal glutamate, glutamine, and GABA levels remains intact during repeated binge toluene inhalation in adolescents.

The acute effect of a single exposure to toluene on cortical glutamate and GABA is age-dependent, with no effects in juvenile and adolescent rats but with significant decreases in levels of both neurotransmitters in adult rats (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). However, repeated exposure to toluene exerted a differential regulation of amino acid neurotransmitters in adolescents that was dependent on the duration of abstinence (Figure 1). Specifically, repeated binge toluene inhalation significantly decreased cortical glutamate but did not affect GABA after 1 day of abstinence. On the other hand, 7 days after the repeated binge toluene inhalation, cortical GLU levels returned to control levels and GABA levels increased. Functionally, a decrease in GLU or an increase in GABA is consistent with a shift away from neuronal excitation toward inhibition, and is also consistent with deficits in excitatory neurotransmission observed in rats after repeated exposure to a variety of addictive drugs (Kalivas, 2009). The observations herein also suggest that the acute effects of a single toluene exposure on cortical glutamate in adult (O'Leary Moore et al., 2009) are mimicked by repeated toluene in the adolescent, an effect that resolves 7 days after drug exposure. Altered levels of cortical GLU and GABA may be related to toluene-induced plasticity of cognate receptor systems, namely the observation that chronic exposure to 8000 ppm of toluene increased GABA-A $\alpha 1$, NR1, NR2B and GluR2/3 receptor subunits in the mPFC (Williams et al., 2005). Clinical research shows that chronic toluene abuse produces neurological and neuropsychological dysfunctions that are correlated with abnormal cortical CAT scans (Fornazzari et al., 1983) or MRI results (e.g., compromised myelin) (Filley et al., 2004; Yucel et al., 2008). The current observations may reveal pre-symptomatic neurochemical alterations and suggest that ^1H -MRS may be useful in the early detection of toluene abuse-induced neural changes in a neurochemical profile.

Toluene regulates the amino acid neurotransmitters and their ionotropic receptors in the hippocampus of rodents. For example, a toluene challenge increases extracellular glutamate and taurine in the hippocampus of adult rats (Win-Shwe et al., 2007) and we found that a single toluene inhalation decreases GLU and GABA in juveniles, decreases GABA but not GLU in adolescents, and has no effect on GLU or GABA in adult rats (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009). Chronic toluene increases NMDA receptor mediated currents, decreases GABA-mediated currents, and increases the levels of NR2A and NR2B receptor subunits in adult rats (Bale et al., 2005b). In the current study, repeated toluene exposure did not significantly affect GLU or GABA in adolescent hippocampus 1 day after the last exposure (Figure 3); although levels of myoinositol, NAA, and taurine were decreased (see Supplement). Decreased NAA and myoinositol after an extended exposure to a potential neurotoxicant is consistent with a frank loss of neurons and glia, respectively. Conversely, 7 days after repeated toluene exposure, hippocampal GLU and GABA levels were increased, suggesting a delayed neuroplasticity in the adolescent hippocampus (Figure 3). The relationship between neurochemical changes and behavioral or functional sequelae associated with the hippocampus remains to be established.

Interpretation of the current significant effects and interactions involving time (i.e., assessments 1 day versus 7 days after ceasing toluene exposure), as well as interpretation of differences in neurochemical responses to toluene across studies with rats at different ages (cf., (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009), must be qualified by another developmental consideration. The post-toluene 7-day period of abstinence coincided

precisely with a critical phase of peri-adolescent brain maturation characterized by changing endocrine and neurochemical function and shifting behavioral and psychopharmacological responsivity (Spear, 2000a; Spear, 2000b; Spear and Brake, 1983). These developmental changes have been quantified using ^1H -MRS and include effects of age on most neurochemical levels including GLU (Burri et al., 1990; Tkac et al., 2003; Yao et al., 1999). It is possible, therefore, that differences between the 1-day and 7-day periods of abstinence are also influenced by – or interacting with – maturational changes that are occurring normally in rats between P35 and P42. The significant decreases between P35 and P42 in the 0-ppm control group in GLU in mPFC, and in glutamine and GABA in hippocampus, for example, reflect these maturational changes and are consistent with a previous report of decreased levels of GLU and GABA between PN21 and adulthood in mice using ^1H -MRS (Yao et al., 1999). However, our conclusions about the effects of toluene at this age take developmental changes into account by including Time as a factor in the analyses.

This study investigated the neurochemical effects of toluene in a preclinical model of binge-pattern inhalation exposure to high-concentration repeated toluene administration in adolescence. The study also investigated the persistence or delayed emergence of toluene-induced neurochemical alterations (Bowen et al., 2006; Cruz and Bowen, 2008). The results imply that toluene-induced alterations in GLU, glutamine or GABA in particular brain regions may be related to the clinical manifestation of toluene abuse in teens.

In conclusion, adolescence is a period of particular susceptibility to the abuse of inhaled organic solvents like toluene. The present and previous (O'Leary-Moore et al., 2007; O'Leary-Moore et al., 2009) results showing differential neurochemical changes in adolescence in response to repeated binge patterns of toluene inhalation are consistent with this notion. The age-dependent toluene induced acute and persistent changes in amino acid neurotransmitters, reflecting differential sensitivity to toluene, may contribute to the particular popularity of solvent abuse among adolescents and the risk of later use and abuse of other substances.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Appendix A. Supplementary data

Supplementary data on brain biochemistry changes produced by toluene can be found in the online version at doi:10.1016/j.drugalcdep.xxxx.xx.xxx.

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Figure 1.

The acute (left) and prolonged (right) effects of chronic toluene exposure on glutamate (A), glutamine (B), and GABA (C) in the medial prefrontal cortex determined by ^1H -MRS. Values are mean ($\pm$ S.E.M.) nmol of amino acid neurotransmitter / mg tissue (* $p < 0.05$).

Anterior Striatum

Figure 2.

The acute (left) and prolonged (right) effects of chronic toluene exposure on glutamate (A), glutamine (B), and GABA (C) in the anterior striatum determined by ^1H MRS. Values are mean ($\pm$ S.E.M.) nmol of amino acid neurotransmitter / mg tissue (* $p < 0.05$).

Hippocampus

Figure 3.

The acute (left) and prolonged (right) effects of chronic toluene exposure on glutamate (A), glutamine (B), and GABA (C) in the hippocampus determined by ^1H MRS. Values are mean ($\pm$ S.E.M.) nmol of amino acid neurotransmitter / mg tissue (* $p < 0.05$).