Title: Chronic intermittent toluene inhalation in adolescent rats alters behavioural responses to amphetamine and MK801

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Abstract

Abuse of toluene-containing inhalants is common during adolescence, with ongoing chronic misuse associated with adverse outcomes and increased risk for addictive behaviours in adulthood. However, the mechanisms mediating the adaptive processes related to these outcomes are not well defined. To model human abuse patterns we exposed male adolescent Wistar rats (postnatal day 27) to chronic intermittent inhaled toluene (CIT, 10,000 ppm) or air (control) for 1 hour/day, 3 times/week for 3 weeks. The effects of CIT on behaviour and recovery were monitored. Locomotor activity was recorded following two consecutive injections of amphetamine (1mg/kg, i.p.) 72 and 96 hours after the last exposure. This was followed with injection of the NMDA receptor antagonist MK801 (0.5mg/kg, i.p.) 20 days after the last exposure. CIT resulted in a significant and persistent retardation in weight gain during the exposure period and abstinence ($p<0.05$). Repeated exposure resulted in tolerance to the onset of toluene-induced behaviours and recovery latency. There was a reduction in the acute stimulant effects of amphetamine in CIT-exposed animals and an increase in the magnitude of locomotor activity ($p<0.0125$) following a subsequent exposure when compared to the responses observed in controls; this was associated with altered locomotor responses to MK801. Repeated exposure to CIT during adolescence alters parameters of growth, as measured by body weight, and leads to tolerance indicating increasing concentrations of the compound may be needed to reach the same behavioural state. Toluene during this period also alters responses to a psychostimulant which may be related to long-term glutamatergic dysfunction.

Keywords*:* Animal model, Inhalant abuse, NMDA, Tolerance

Introduction

The abuse of inhaled chemical vapours is a significant public health concern especially among adolescent populations (National Drug Survey, 2011). This is in part because inhalants are cheap, legal, accessible and provide a rapid 'high'. Chronic abuse of inhalants during adolescence is related to altered cognitive function, decreased IQ, anxiety, depressive symptoms and increased impulsivity (Perron and Howard, 2009), with many experiencing major psychiatric problems and/or progressing to illicit drug use (Johnson et al., 1995; Perron and Howard, 2009). These long-term impacts are hypothesized to occur due to the vulnerability of the immature brain as processes such as myelination and structural reorganization are occurring (Lubman et al., 2008). Consequently, as shown in rodents, adolescents display different sensitivities to inhalants than adults (Bowen et al., 2007a) and may be more susceptible to injury and/or long-term neuroadaptations following inhalant misuse.

The volatile solvent toluene (methyl benzene) is found in many household products including paints, thinners, aerosols and petrol and has high abuse potential due to its ability to modulate neural reward pathways including dopaminergic processes (Riegel et al., 2007). Increasing evidence also suggests that toluene may have long-lasting effects on the gamma-aminobutyric acid (GABA)ergic and glutamatergic systems, the latter occurring primarily via interactions with N-methyl D-aspartate (NMDA) receptors (Beckstead et al., 2000; Cruz et al., 1998). Thus, repeated exposure to toluene may lead to neuroadaptive processes in these systems that help explain the adverse outcomes seen in human abusers, including an increased risk for subsequent drug use in adulthood. However, whether repeated exposures results in altered sensitivity (tolerance or cross-sensitization) to subsequent exposures to either toluene itself or

other drugs remains inconclusive (see Bowen and Balster, 2006 for review). Thus, we hypothesise that chronic intermittent exposure to toluene during adolescence will result in adaptive processes that alter the sensitivity of the brain to subsequent exposures to toluene or another drug of abuse such as amphetamine and that this will in part be mediated by long-term NMDA receptor mediated glutamatergic dysfunction.

Experimental Procedures

Animals

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia. Adolescent male Wistar rats (~postnatal day (PND) 24 –where adolescence ranges from PND 21 to 60; see Duncan et al., 2012) were obtained from the Australian Resources Centre (Perth, Australia). Rats were pair housed, maintained on a 12-hour light/dark cycle with food and water *ad libitum*. Rats were acclimatised for 3 days prior to experimentation.

Toluene Inhalation Exposure

Exposure to 10,000ppm vapourised liquid toluene (n=8, Merck, Vic, Australia) was conducted as previously described (Duncan et al., 2012) and the concentration (10,000±100ppm) of toluene verified using a calibrated inline gas chromatography system (Shimadzu Corporation, Kyoto, Japan). Replica chambers exposed to room air were utilised for control animals (n=7).

During a 1 hour acclimation period body weights were recorded. Rats were exposed to chronic intermittent toluene (CIT) or air for 1hr/day, 3 times/week (Monday, Wednesday, Friday), for 3 weeks (9 sessions total ranging from PND 27-45). This exposure paradigm was employed to reflect the human pattern of toluene abuse at a concentration relevant to the human situation (see Lubman et al., 2008). To avoid any possible confounds of olfactory stimulation in control animals due to toluene sticking to the fur of CIT-exposed rats, rats were placed back into

their home cage and semi-isolated for 1hr. Exposures were conducted at room temperature (~21°C) under normal lighting.

Acute Behaviour and Recovery

In rats exposed to CIT (n=8), animals were filmed while in the chamber during their first and last exposure and the latency to engage in two toluene induced behaviours (loss of hind limb coordination and loss of movement) recorded. Immediately after exposure rats were placed in locomotor cells (40min) to record horizontal (HP) and vertical plane (VP) activity (TruScan^{TM} Photobeam; Coulbourn Instruments, Allentown, PA, USA).

Post-Exposure Drug Challenge

To investigate whether CIT alters responses to a psychostimulant, 3 days after the last exposure animals were injected with amphetamine (1mg/kg, i.p.) and locomotor activity recorded (both HP and VP activity) for 2 hours (following 30min habituation to the locomotor cells). To assess the response following a subsequent exposure to the same drug locomotor activity was also recorded the following day after a second administration of amphetamine (1mg/kg, i.p.). To assess long-term glutamatergic dysfunction, locomotor activity was measured following an i.p injection of MK801 (0.5mg/kg) 20 days after the last exposure to air or CIT under the same conditions. Amphetamine was administered 3 days after the last exposure to ensure sufficient time had passed for toluene to be metabolised and cleared from the body and to reflect a different drug exposure at the same time point when animals would normally receive toluene. MK801 was injected 20 days after the last exposure (P65) to investigate long-term glutamatergic changes maintained into adulthood.

Statistical Analysis

Body weights (exposure period) and locomotor activity during recovery or VP data (post-exposure drug challenge) were analysed using a 2-way repeated-measures (RM) analysis of variance (ANOVA) with "time" and "treatment" as factors. Post-hoc analysis was undertaken using the Holm-Sidak all pairwise multiple comparisons. Post-exposure drug challenge data (HP) were analysed using a 3-way RM ANOVA for the variable "distance travelled" with the repeated factors "post-exposure drug treatment" and "time" and the between factor "pre-treatment" (air or toluene). To determine where differences existed, post-hoc analysis for responses to amphetamine (across days or within pretreatment) or MK801 were analysed using a 2-way RM ANOVA and the Holm-Sidak all pairwise multiple comparisons. Body weights after 20 days abstinence and total VP entries (recovery and MK801) were analysed using a student's *t*-test. All statistical analyses were performed using SigmaStat 3.5 (Jandel, San Jose, CA, USA) or SPSS (3-way RM ANOVA, IBM Software, NSW, Aust.). Graphs were produced using GraphPad Prism 5 (San Diego, CA, USA). Data are presented as mean ± SEM; recovery locomotor data are presented in 4min time bins (40min total) and post-exposure drug challenge data presented in 5min time bins (150min total including 30mins habituation). Statistical significance was accepted at $p \leq 0.05$ except for post-exposure drug challenge data where statistical significance was accepted at $p \leq 0.0125$ to correct for multiple testing.

Results

Effects of CIT Exposure on Growth

Exposure to CIT during adolescence resulted in a significant retardation in body weight gain during this period. A 2-way RM ANOVA revealed a main effect of time ($F_{(8,104)}$=2063.3, $p<0.001$) and treatment ($F_{(1,104)}$=12.2, $p=0.004$) on body weight throughout the exposure period, and a significant time x treatment interaction ($F_{(8,104)}$=26.2, $p<0.001$). Post-hoc analysis revealed that CIT-exposed animals had significantly reduced body weights by PND 36 (after 4 exposures) compared to controls, with a 14.3% reduction by the last exposure (Figure 1A). The effects of CIT on body weight were still evident after 20 days of abstinence (15.2% reduction, $p=0.0002$, *t*-test, Figure 1B).

Acute Behaviour and Recovery

On the first exposure to CIT animals lost hind limb coordination within 4mins and total body movement within 8mins (Figure 2A, B). By the final exposure to CIT (session 9) there was a significant increase in the latency for both of these behaviours ($p<0.0001$ and $p=0.013$ respectively, *t*-test, Figure 2A, B). Following the first exposure to toluene, a significant increase in HP locomotor activity was not present until 32mins into recovery. By the final exposure (session 9) HP activity recovered faster being significantly increased by 20mins (main effect of time, $F_{(9,126)}$=8.8, $p<0.001$, RM ANOVA)(Figure 2C). There were no differences in total VP entries during recovery between the first and last exposure to CIT (0.25 ± 0.71 vs 3.75 ± 5.50 entries respectively, *t*-test).

Post-Exposure Drug Challenge

Chronic intermittent exposure to toluene during adolescence altered locomotor (HP) responses to subsequent exposures to drugs during late-adolescence and early adulthood. A 3-way RM ANOVA revealed a main effect of post-exposure drug treatment ($F_{(2,26)}$=13.7, p<0.0001) and time ($F_{(29,377)}$=19.3, p<0.0001) and significant interactions between post-exposure drug treatment x time ($F_{(58,754)}$=5.9, p<0.0001), pre-treatment x time ($F_{(29,377)}$=3.4, p<0.0001) and pre-treatment, post-exposure drug treatment and time ($F_{(58,754)}$=3.0, p<0.0001).

Amphetamine

Within day analysis: There was no difference in HP locomotor activity between groups during the 30min habituation period. A 2-way RM ANOVA revealed a main effect of time ($F_{(29,377)}$=12.6, p<0.001) and a significant interaction between time x treatment ($F_{(29,377)}$=22.1, p<0.001) following exposure to amphetamine 3 days after the final exposure to CIT. Amphetamine induced HP locomotor responses were reduced in CIT-exposed animals compared to air controls. Following a subsequent exposure to amphetamine 24 hours later there was a main effect of time ($F_{(29,377)}$=20.9, p<0.001, 2-way RM ANOVA) but no difference between groups (Figure 3A, B).

Within treatment analysis: In air-exposed animals HP activity was increased following the second exposure to amphetamine, however a 2-way RM ANOVA revealed a main effect of time ($F_{(29,348)}$=15.9, p<0.001) only (Figure 3A). Within animals previously exposed to CIT a 2-way RM ANOVA revealed a main effect of time ($F_{(298,406)}$=15.7, p<0.001) and a significant time x treatment interaction ($F_{(29.406)}$=3.8, p<0.001). Post-hoc analysis revealed that HP activity was significantly increased in CIT-exposed animals within 10 minutes relative to the responses

following the first treatment (Figure 3B). Analysis of total VP entries indicated a main effect of time ($F_{(1,13)}$=12.4, p=0.004, 2 way RM ANOVA) with post-hoc analysis indicating VP activity was not different across days in animals exposed to air (Day 1: 255 ± 53 vs Day 2: 403 ± 68 entries) but was significantly increased (p=0.009) in animals previously exposed to CIT (Day 1: 133 ± 22 vs Day 2: 353 ± 95 entries).

MK801

There was no difference in habituation, but a differential response in HP locomotor activity between groups following MK801 administered 20 days after the last exposure (Figure 3C). A 2-way RM ANOVA revealed a main effect of time ($F_{(29,377)}$=8.9, p<0.001) as well as a significant time x treatment interaction ($F_{(29,377)}$=3.6, p<0.001). Post-hoc analysis revealed a significant increase (p<0.0125) in HP activity in CIT-exposed animals compared to air controls 15mins post injection. This was followed by a rapid decrease (p<0.0125) in locomotor activity at 30mins which remained suppressed for the next 15mins before returning to control levels (Figure 3C). Total VP entries during this period were not different between groups (air: 61 ± 14 vs CIT: 41 ± 10 entries, *t*-test).

Discussion

We exposed rats to CIT at a concentration abused by humans, at a time point equivalent to early adolescence to reflect the age at which many young people experiment with inhalants (see Lubman et al., 2008). Exposure to CIT had a significant effect on body weight and resulted in tolerance to both the acute behavioural effects and recovery. CIT during adolescence also altered the magnitude of locomotor activity following repeated injections of amphetamine and resulted in persistent glutamatergic dysfunction.

Not unexpectedly, from PND 27 there was an increase in the body weight of all rats. However in animals exposed to CIT this increase was significantly retarded; the difference being observed after only 4 exposures. This concurs with the observation that human adolescent abusers often experience weight loss (Glaser and Massengale, 1962) or present as emaciated (Ryu et al., 1998). The mechanisms mediating these changes are currently unknown but may relate to toluene induced alterations in body composition, nutrient absorption or metabolism (Jarosz et al., 2008).

In human abusers the initial effects of inhalants occur within minutes and are characterized by euphoria, slurred speech, dizziness and ataxia leading to unconsciousness with continued exposure or increasing concentration (Lubman et al., 2008). Acute exposure to CIT in adolescent rats at the concentration used in this study resulted in a loss of motor control and eventual sedation in line with data from human studies (see Lubman et al., 2008), with animals remaining sedated for up to 30 minutes after exposure ceased. Repeated exposure to CIT during adolescence resulted in the appearance of tolerance, as indicated by an increase in the latency for acute behaviours to appear and a more rapid return of locomotor activity during recovery.

Tolerance has been reported in adolescent human abusers who indicate needing to inhale more product to reach the feeling of euphoria felt during initial experimentation (Glaser and Massengale, 1962). It is hypothesized that the appearance of tolerance following repeated exposure to inhalants occurs due to a loss of reinforcement as tolerance on certain measures of performance on a signal detection task including accuracy and increased response failures is present (Oshiro et al., 2007). Adaptive mechanisms in the liver, for example, may also play a role in the appearance of tolerance as enhanced liver metabolism following repeated exposures may lower the concentration of toluene in the body (Elovaara et al., 1979). However, data from animal studies have reported varied findings including both tolerance and cross-sensitization following repeated exposure to toluene (see Bowen and Balster, 2006 for review).

In our paradigm we observed no difference in basal locomotor activity between air and CIT-exposed animals. Following the first amphetamine challenge there was a reduction in psychostimulant induced hyperactivity in CIT-animals relative to controls. Prenatal exposure to toluene (up to 12,000ppm) results in hyposensitivity to the acute stimulatory effects of amphetamine when administered during adolescence (Bowen et al., 2007b) or adulthood (Bowen et al., 2009). However cross-sensitization occurs to cocaine (both induced locomotor activity and accumbal dopamine levels) following 10 days (30min/day) of exposure to 8,000ppm toluene in adult rats (Beyer et al., 2001) suggesting age, drug and/or paradigm-specific effects on factors mediating tolerance versus sensitization following exposure to toluene.

Following repeated exposure to amphetamine there was no difference between CIT-exposed animals and controls suggesting a normalization of locomotor responses. As such there was an increase in the magnitude of difference between the first and second exposure of stimulant induced locomotor activity in CIT animals when compared to controls. To our

knowledge this is the first paper describing a difference in the magnitude of locomotor responses to amphetamine induced by prior exposure to toluene. Oral administration of toluene for 7 days induced behavioural sensitization to toluene, but did not result in cross-sensitization to a subsequent exposure of amphetamine (Wiaderna and Tomas, 2000). Also toluene, at concentrations ranging from 500-6000ppm, partially substituted for amphetamine in a discriminative stimulus paradigm (Bowen, 2006), the greatest affects being observed at the highest concentration. The effects of toluene on behavioural responses to amphetamine are likely to occur via similar actions on the dopaminergic system between the two drugs. Indeed, toluene injected directly into the ventral tegmental area increases neuronal firing and dopamine release in the nucleus accumbens (Riegel et al., 2007), with chronic exposure leading to persistent dopaminergic dysfunction (Hillefors-Berglund et al., 1995).

We observed a difference in HP activity in animals challenged with MK801 20 days after the last CIT exposure, initial hyperlocomotion followed by hypolocomotion (stereotypies) relative to controls. This would suggest persistent dysfunction of NMDA receptor signaling. In line with this observation experiments using transfected *Xenopus* oocytes (Cruz et al., 1998) or whole-cell patch clamping of medial prefrontal cortex neurons (Beckley and Woodward, 2011) have shown that exposure to toluene dose-dependently inhibits NMDA-mediated currents in a rapid and reversible manner with the GluN1/2B subunit being most sensitive (Cruz et al., 1998). Furthermore, Bale et al. (2007) have shown interactions between toluene and MK801 involve NMDA receptors. Thus, the altered response in the current study may suggest functional antagonism between these two compounds on NMDA-mediated responses. However toluene does not substitute for MK801 in a self-administration paradigm in mice suggesting that NMDA receptor antagonism is not the sole factor contributing to, at least, altered operant responding

(Shelton and Balster, 2004). Indeed toluene's effects are far reaching, in the medial prefrontal cortex changes to NDMA receptors occur in association with a decrease in 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) currents and potentiation of $GABA_A$ currents (Beckley and Woodward, 2011). This is complicated by the fact that NMDA and GABA mediated responses are reversed following prolonged exposure to toluene (Bale et al., 2005b) and toluene is known to also modulate other neurotransmitters including the cholinergic (Bale et al., 2005a) and serotonergic systems (Lopreato et al., 2003).

While our most robust finding of the effects of CIT were observed following subsequent exposure to MK801 it is worth noting that amphetamine is an indirect dopamine agonist and changes to locomotion following MK801 are in part driven by dopaminergic mechanisms. Thus further exploration is needed to fully determine whether toluene-induced changes are mediated by long-term dopaminergic or glutamatergic dysfunction, or both. Indeed these systems and the effects on different elements of behaviour are likely to be highly interrelated as demonstrated by Lo and colleges using antagonist and agonist studies (Lo et al., 2009).

We conclude that exposure to CIT during adolescence at concentrations relevant to human use has a direct effect on body weight and results in adaptive changes including tolerance and altered responses to drugs of abuse. While the exact mechanisms are yet to be fully elucidated, our data suggest that these responses may, in part, be mediated by persistent changes to NMDA receptor mediated signaling.

References

2010 National Drug Strategy Household Survey report, Australian Institute of Health and Welfare, Canberra, Aust. 260 pages.

Bale, A.S., Jackson, M.D., Krantz, Q.T., Benignus, V.A., Bushnell, P.J., Shafer, T.J., Boyes, W.K., 2007. Evaluating the NMDA-glutamate receptor as a site of action for toluene, *in vivo*. Toxicol. Sci. 98, 159-166.

Bale, A.S., Meacham, C.A., Benignus, V.A., Bushnell, P.J., Shafer, T.J., 2005a. Volatile organic compounds inhibit human and rat neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes. Toxicol. Appl. Pharmacol. 205, 77-88.

Bale, A.S., Tu, Y., Carpenter-Hyland, E.P., Chandler, L.J., Woodward, J.J., 2005b. Alterations in glutamatergic and gabaergic ion channel activity in hippocampal neurons following exposure to the abused inhalant toluene. Neurosci. 130, 197-206.

Beckley, J.T., Woodward, J.J., 2011. The abused inhalant toluene differentially modulates excitatory and inhibitory synaptic transmission in deep-layer neurons of the medial prefrontal cortex. Neuropsychopharm. 36, 1531-1542.

Beckstead, M.J., Weiner, J.L., Eger, E.I., 2nd, Gong, D.H., Mihic, S.J., 2000. Glycine and gamma-aminobutyric acid(A) receptor function is enhanced by inhaled drugs of abuse. Mol. Pharmacol. 57, 1199-1205.

Beyer, C.E., Stafford, D., LeSage, M.G., Glowa, J.R., Steketee, J.D., 2001. Repeated exposure to inhaled toluene induces behavioral and neurochemical cross-sensitization to cocaine in rats. Psychopharmacol. 154, 198-204.

Bowen, S.E., 2006. Increases in amphetamine-like discriminative stimulus effects of the abused

inhalant toluene in mice. Psychopharmacol. 186, 517-524.

Bowen, S.E., Balster, R.L., 2006. Tolerance and sensitization to inhaled 1,1,1-trichloroethane in mice: results from open-field behavior and a functional observational battery. Psychopharmacol. 185, 405-415.

Bowen, S.E., Charlesworth, J.D., Tokarz, M.E., Wright, M.J., Jr., Wiley, J.L., 2007a. Decreased sensitivity in adolescent vs. adult rats to the locomotor activating effects of toluene. Neurotoxicol. Teratol. 29, 599-606.

Bowen, S.E., Hannigan, J.H., Cooper, P.B., 2009. Abuse pattern of gestational toluene exposure alters behavior in rats in a "waiting-for-reward" task. Neurotoxicol. Teratol. 31, 89-97.

Bowen, S.E., Mohammadi, M.H., Batis, J.C., Hannigan, J.H., 2007b. Gestational toluene exposure effects on spontaneous and amphetamine-induced locomotor behavior in rats. Neurotoxicol. Teratol. 29, 236-246.

Cruz, S.L., Mirshahi, T., Thomas, B., Balster, R.L., Woodward, J.J., 1998. Effects of the abused solvent toluene on recombinant N-methyl-D-aspartate and non-N-methyl-D-aspartate receptors expressed in Xenopus oocytes. J. Pharmacol. Exp. Ther. 286, 334-340.

Duncan, J.R., Dick, A.L., Egan, G., Kolbe, S., Gavrilescu, M., Wright, D., Lubman, D.I., Lawrence, A.J., 2012. Adolescent toluene inhalation in rats affects white matter maturation with the potential for recovery following abstinence. PLoS One 7, e44790.

Elovaara, E., Savolainen, H., Pfaffli, P., Vainio, H., 1979. Effects of subacute toluene inhalation on its metabolism and disposition in rat. Arch. Toxicol. Suppl. 2, 345-348.

Glaser, H.H., Massengale, O.N., 1962. Glue-sniffing in children. Deliberate inhalation of vaporized plastic cements. JAMA. 181, 300-303.

Hillefors-Berglund, M., Liu, Y., von Euler, G., 1995. Persistent, specific and dose-dependent

effects of toluene exposure on dopamine D2 agonist binding in the rat caudate-putamen. Toxicol. 100, 185-194.

Jarosz, P.A., Fata, E., Bowen, S.E., Jen, K.L., Coscina, D.V., 2008. Effects of abuse pattern of gestational toluene exposure on metabolism, feeding and body composition. Physiol. Behav. 93, 984-993.

Johnson, E.O., Schutz, C.G., Anthony, J.C., Ensminger, M.E., 1995. Inhalants to heroin: a prospective analysis from adolescence to adulthood. Drug and alcohol dependence 40, 159-164.

Lo, P.S., Wu, C.Y., Sue, H.Z., Chen, H.H., 2009. Acute neurobehavioral effects of toluene: involvement of dopamine and NMDA receptors. Toxicol. 265, 34-40.

Lopreato, G.F., Phelan, R., Borghese, C.M., Beckstead, M.J., Mihic, S.J., 2003. Inhaled drugs of abuse enhance serotonin-3 receptor function. Drug Alcohol Depend. 70, 11-15.

Lubman, D.I., Yucel, M., Lawrence, A.J., 2008. Inhalant abuse among adolescents: neurobiological considerations. Br. J. Pharm. 154, 316-326.

Oshiro, W.M., Krantz, Q.T., Bushnell, P.J., 2007. Repeated inhalation of toluene by rats performing a signal detection task leads to behavioral tolerance on some performance measures. Neurotoxicol. Teratol. 29, 247-254.

Perron, B.E., Howard, M.O., 2009. Adolescent inhalant use, abuse and dependence. Addiction 104, 1185-1192.

Riegel, A.C., Zapata, A., Shippenberg, T.S., French, E.D., 2007. The abused inhalant toluene increases dopamine release in the nucleus accumbens by directly stimulating ventral tegmental area neurons. Neuropsychopharmacol. 32, 1558-1569.

Ryu, Y.H., Lee, J.D., Yoon, P.H., Jeon, P., Kim, D.I., Shin, D.W., 1998. Cerebral perfusion impairment in a patient with toluene abuse. J. Nucl. Med. 39, 632-633.

Shelton, K.L., Balster, R.L., 2004. Effects of abused inhalants and GABA-positive modulators in dizocilpine discriminating inbred mice. Pharmacol. Biochem. Behav. 79, 219-228.

Wiaderna, D., Tomas, T., 2000. Effects of repeated exposure to toluene or amphetamine on locomotor activity in rats. Int. J. Occup. Med. Environ. Health. 13, 317-324.

Figure Legends

Figure 1. Effects of CIT on body weight. A) Body weight (g) throughout the 3 week exposure period to CIT (n=8) or air (n=7) beginning postnatal day (PND) 27 or B) following a subsequent period of 20 days of abstinence (PND 65). Data are expressed as mean (± SEM). *p<0.05 difference between groups (2-way RM ANOVA with Holm–Sidak post-hoc tests) or **p=0.0002 (*t*-test).

Figure 2. Acute Behaviour and Recovery. The latency (min) to display acute behaviours including loss of A) hind limb coordination and B) total movement during the initial and final exposure to CIT. C) Recovery of horizontal plane locomotor activity (cm) after the initial and final exposure to CIT. Data are expressed as mean (± SEM), locomotor data measured in 4 minute time bins, n=8. *p<0.02, **p<0.001 (*t*-test) or #p<0.05 difference in time (2-way RM ANOVA with Holm–Sidak post-hoc tests).

Figure 3. Post Exposure Drug Challenge. Horizontal plane locomotor activity (cm) following exposure to amphetamine (1mg/kg, i.p.) 72 or 96 hours after the last exposure to air or CIT. Data are presented as responses in A) air controls (n=7) or B) CIT-exposed animals (n=8) across the first and second exposure to amphetamine. C) Horizontal plane locomotor activity (cm) following exposure to MK801 (0.5mg/kg, i.p.) 20 days after last exposure to air (n=7) or CIT (n=8). Data are expressed as the mean (± SEM) measured in 5 minute time bins. *p<0.0125 main effect of day (B) or treatment (C); main effect of time present in all graphs (2-way RM ANOVA with Holm–Sidak post-hoc tests). Line at 30 minutes indicates point of injection.

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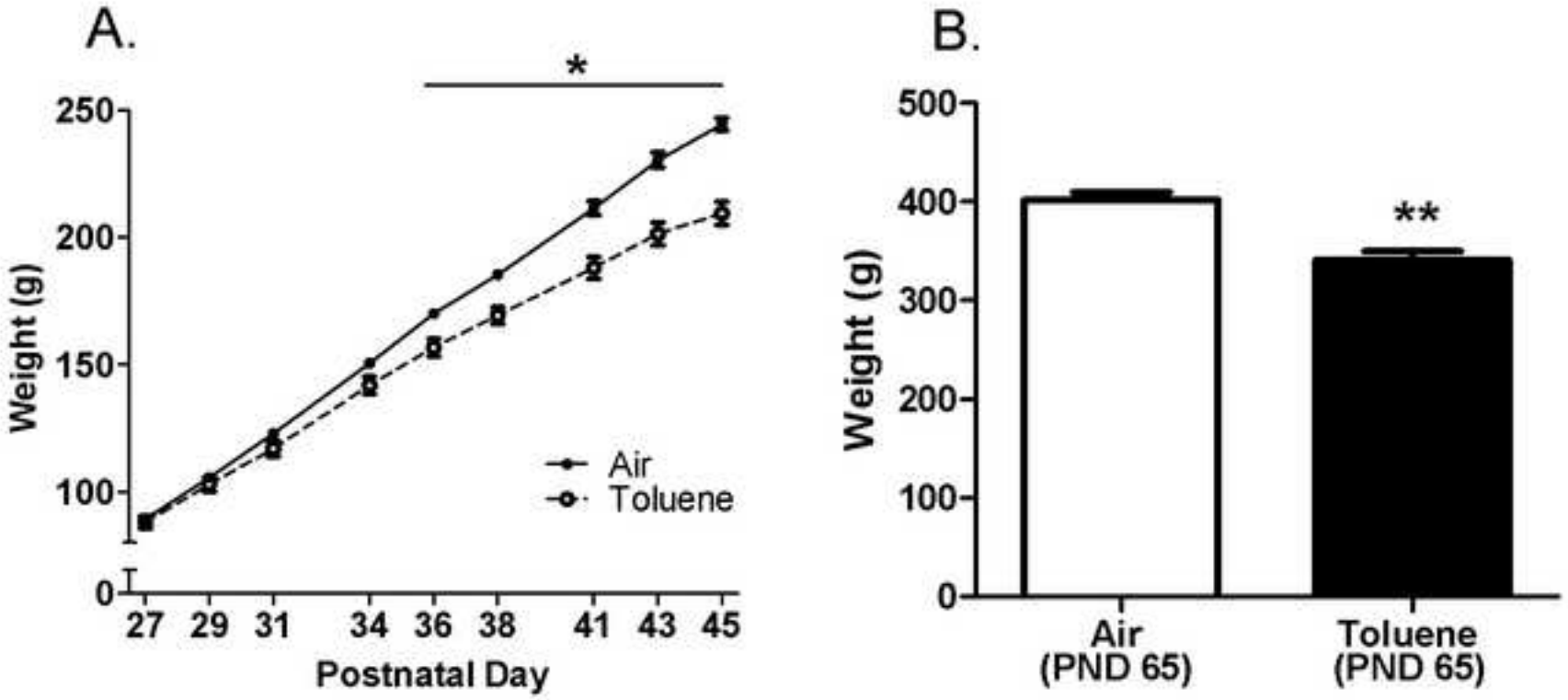

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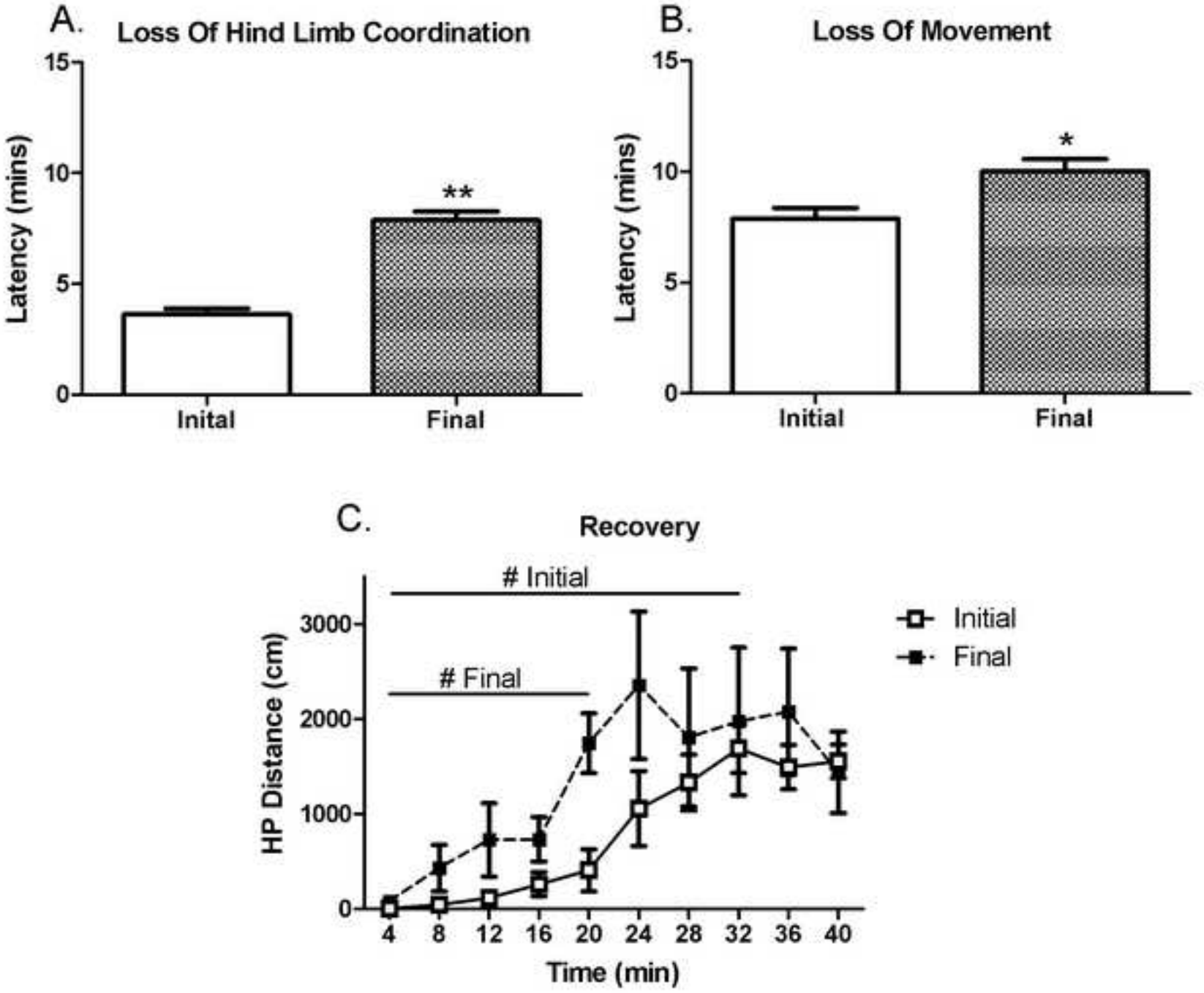

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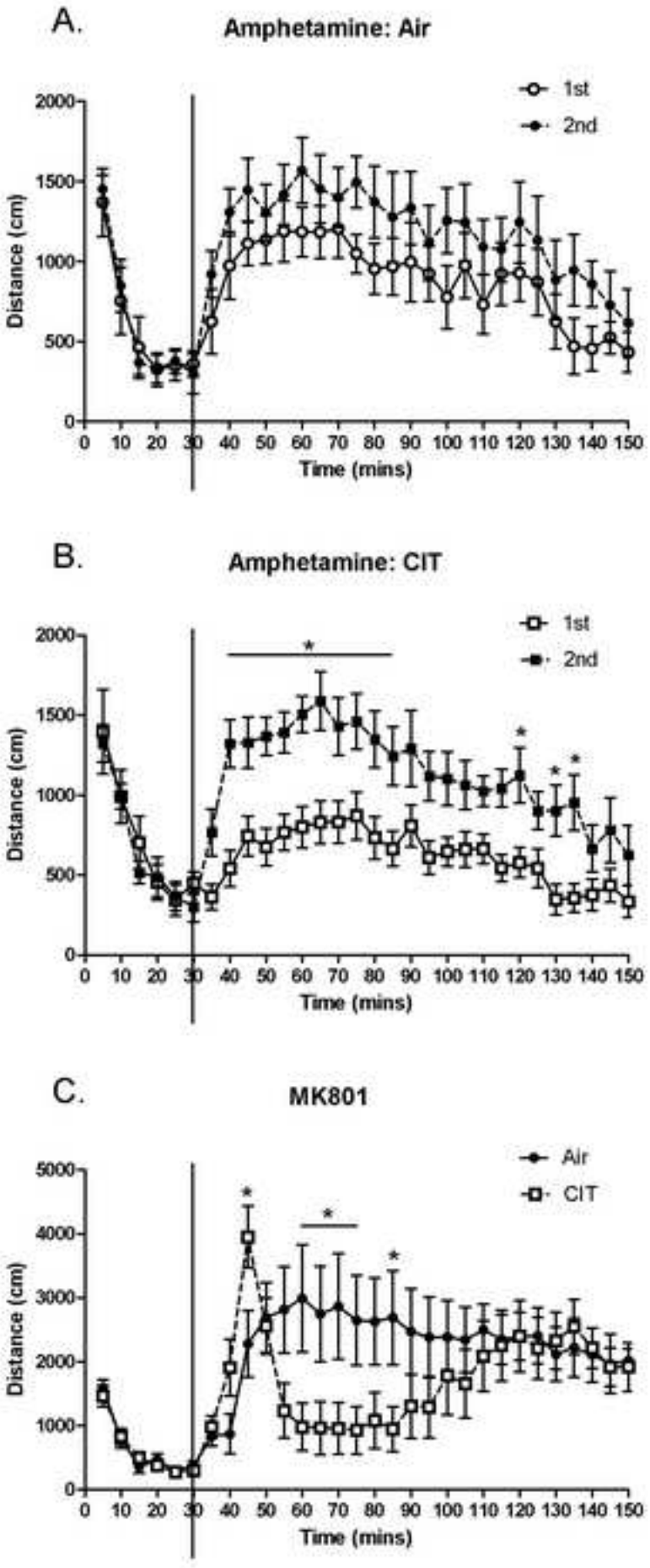

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Contributors: Author Duncan was involved in the study design, development of experimental protocols, supervision of experiments and wrote the first draft of the manuscript. Author Gibbs was involved in carrying out the experiment, data analysis and interpretation. Author Lawrence was involved in study design, data interpretation and drafting of the manuscript. All authors have contributed to and have approved the final manuscript

Author Disclosures

Conflict of interest: All authors declare that they have no conflicts of interest.

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