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Comparison of toluene-induced locomotor activity in four mouse strains ☆

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ABSTRACT

The mechanisms by which abused inhalants exert their neurobehavioral effects are only partially understood. In research with other drugs of abuse, specific inbred mouse strains have been useful in exploring genetic loci important to variation in behavioral reactions to these drugs. In the present investigation, mice from three inbred strains (Balb/cByj, C57BL/6J and DBA/2J) and one outbred strain (Swiss Webster) were studied for their acute and chronic sensitivity to toluene-induced changes in locomotor activity. Mice were exposed to toluene (0, 100, 2000, 8000, and 10,000 ppm) for 30 min in static exposure chambers equipped with activity monitors. In the acute condition, concentrations of toluene <8000 ppm increased ambulatory distance while the concentrations of ≥8000 ppm induced temporally biphasic effects with initial increases in activity followed by hypoactivity. Between-group differences in absolute locomotor activity levels were evident. The inbred Balb/cByj and DBA/2J strains as well as the outbred Swiss Webster strain of mice showed greater increases in activity after an acute challenge exposure to 2000 ppm than the inbred C57BL/6J strain. The same animals were then exposed 30 min/day to 8000 ppm toluene for 14 consecutive days. Re-determination of responses to 2000-ppm challenge exposures revealed that sensitization developed in locomotor activity and that the DBA/2J strain showed the greatest increase in sensitivity. These baseline differences in acute sensitivity and the differential shifts in sensitivity after repeated exposures among the inbred mouse strains suggest a genetic basis for the behavioral effects to toluene. The results support the notion that like for other drugs of abuse, using various strains of mice may be useful for investigating mechanisms that underlie risk for inhalant abuse.

1. Introduction

Inhalant abuse continues to be a complicated and potentially life-threatening worldwide public health problem (NIDA, 2005). The inhalation of particular volatile chemicals found in common household products like adhesives or cleaning fluids can lead to dizziness, disinhibition, euphoria, slurred speech, ataxia and even death (Bowen et al., 1999; Flanagan and Ives, 1994; Kurtzman et al., 2001). Young people continue to be at risk because solvents are still legal, easily accessible, and inexpensive throughout most areas of the world. Inhalant use in the United States and other countries remains a serious and costly epidemic. As reported in the most recent National Survey on Drug Use and Health, more than 775,000 individuals aged 12 or older that had used inhalants for the first time within the past year (SAMHSA, 2008) with about two-thirds of these individuals being under the age of 18 when they first inhaled these substances. In addition, the latest *Monitoring the Future* study revealed that the percentage of 8th graders who thought it was dangerous to try inhalants even once or twice has decreased over the last couple of years to only 33.9% (Johnston et al., 2008). Within this general trend of greater inhalant drug abuse, over 30% of youths reported using inhalants containing toluene (SAMHSA, 2006). While inhalant use among youth remains relatively high, the individual differences in behavioral and neural outcomes following acute exposures to organic solvents like toluene are poorly understood.

For the general clinical population, individual differences exist in the kind and/or magnitude (i.e., sensitivities) of responses to drugs which may influence the liability to abuse and dependence on these drugs. While many may try a drug once or twice, only a small percentage of those individuals continue with long-term drug taking behaviors. The risk for abuse can be affected by environmental factors such as poverty, stress, abuse and/or lack of good role models, and can also be influenced by genetic factors. While inherited contributions are known to be important, relatively little is known of the genetics that affect drug abuse. For example, in humans the risk of alcoholism is known to be influenced by aldehyde dehydrogenase variants, with

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each giving variable levels of protection (Enoch and Goldman, 2001), and in rats and mice, selective breeding (e.g., LS/SS) can profoundly affect physiological and behavioral responses to alcohol as well as drinking behavior (see review Erwin and McClearn, 1981). Several laboratory studies have shown that genetic background can markedly alter the reinforcing effects to drugs of abuse (Crabbe et al., 1994; Crawley et al., 1997), a hypothesis that is also supported in human populations (Agrawal and Lynskey, 2008; Sartor et al., 2008, 2009; Sullivan and Kendler, 1999). Family studies of adopted children and twins have also suggested that genetic factors can influence the vulnerability to various drugs of abuse (Cadoret et al., 1986; Luthar and Rounsaville, 1993). Preclinical studies using inbred rodent strains have shown that genetic factors can differentially influence responses to a number of drugs of abuse. These findings all support the notion that genotype — at least as reflected in strain — is an important factor influencing individual differences in sensitivity and vulnerability to substances of abuse (Crabbe et al., 1994).

Inbred mouse strains have been valuable tools for exploring the genetic bases of complex behaviors. For example, differences have been observed for baseline locomotor activity among several inbred strains (Crawley et al., 1997; DeFries et al., 1978; Henderson, 1967). The profile of drug-induced locomotor stimulation has been shown to be influenced by the strain of mouse used for cocaine (George, 1989; Morse et al., 1993, 1995; Ruth et al., 1988; Tolliver and Carney, 1994a, b; Wiener and Reith, 1990), ethanol (Lessov et al., 2001; Palmer et al., 2002a,b; Quertemont et al., 2004; Tambour et al., 2006, 2007) and morphine (Gill and Boyle, 2008). While dose-dependent differences in locomotor activation are common among inbred strains of mice in response to a number of abused substances, differences in sensitivity and/or vulnerability to effects of inhaled organic solvents like toluene have not been assessed.

Recent animal laboratory studies of the effects of abused inhalants have been invaluable in defining biobehavioral profiles of inhalant effects (see reviews (Bowen et al., 2006; Evans and Balster, 1991). In particular, a number of studies demonstrated that several abused inhalants have effects similar to central nervous system depressants, like ethanol. Like for other drugs of abuse, acute inhalant exposure produces changes in animal behavior that are concentration-dependent, reversible, and occur at blood levels which are much lower than those necessary to produce explicit toxicological signs (Bowen et al., 2006; Evans and Balster, 1991). The present study was conducted to examine the effects of acute and repeated administration of inhaled toluene on locomotor activity in several strains of mice. Mice from three inbred strains (Balb/cByj, C57BL/6J and DBA/2J), and one outbred strain (Swiss Webster) were used in the present study. These inbred strains were chosen because of their differential responses to other drugs of abuse such as ethanol (Crabbe et al., 1994, 2005; Phillips, 1997). The outbred Swiss Webster strain was included because it represents a genetically heterogeneous stock of mice and it has been the strain most frequently used in earlier reports examining the behavioral effects of solvent exposure (Bowen et al., 2006; Cruz and Bowen, 2008; Evans and Balster, 1991). Finally, we also assessed performance in this task after repeated toluene exposures because repeated solvent exposure is known to alter behavioral responses to subsequent solvent exposures (Bowen and Balster, 2006; Hinman, 1987).

2. Method

All animal procedures had prior approval by the Wayne State University Institutional Animal Care and Use Committee and were conducted in accordance with the NIH "Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Academy Press 1996; NIH Publication No. 85-23, revised 1996).

2.1. Subjects

Adult inbred male mice (Balb/cByj, DBA/2J; $N=11$ per strain and C57BL/6J; $N=9$) and the adult outbred male Swiss Webster mice ($N=11$) were purchased from Charles Rivers Breeding Laboratories (Portage, MI, USA). Mice were housed in groups of 3–4 in polypropylene cages (18 cm × 29 cm × 13 cm) with hardwood chip bedding and steel-wire tops. Mice were allowed *ad lib* access to Rodent Lab Diet 5001 (PMI, Nutrition International, Inc., Brentwood, MO) and water when in their home cages. The AAALAC-approved vivarium was kept on a 12-h light/12-h dark cycle (lights on 0600 h). Animals were transported to the laboratory for all behavioral testing which was carried out during the light cycle.

2.2. Inhalation exposure procedures

Exposure procedures were identical to those previously described (Bowen et al., 2006). Briefly, vapor exposures were given in one of 3 sealed 36-liter cylindrical glass jars housed within a fume hood. During exposure, one mouse was placed into the chamber onto a grid floor 20 cm from the bottom and 30 cm from a vapor diffuser with fan in the Plexiglas lid. For air-only exposures, the lid was sealed (with nothing injected into the diffuser) and the fan turned on. For toluene exposures, a calculated amount of solvent was injected into the diffuser and the fan turned on to volatilize and distribute the toluene within the exposure chamber. Toluene vapor concentrations were confirmed by single wavelength-monitoring infrared spectrometry (Miran 1A, Foxboro Analytical). Mean concentrations of toluene were within 3% of nominal levels ~2.5 min after the solvent was added, and remained within 2% of nominal throughout the 30-min exposure. Levels of waste gases (i.e., water vapor and CO_2) had been monitored during previous studies and changes during 30-min sessions were negligible. After the 30-min exposure, the lids were unsealed and the mice were allowed to recover. After ~60 min, mice were returned to their home cages.

2.3. Study design

2.3.1. Phase 1: initial concentration–effect determination

An acute concentration–effect determination was assessed for the effects of toluene on locomotor activity. Following a 5-min acclimation, mice were given 30-min vapor exposures to toluene concentrations of 100, 2000, 8000, or 10,000 ppm and the appropriate air control (0 ppm) administered in a counterbalanced order on Tuesdays and Fridays over 18 days. (This "acute" or "Pre-chronic" exposure phase of the study was completed before the repeated, i.e., so-called "Post-chronic" exposure phase). The concentrations of toluene selected for acute administration were based on previous investigations of toluene in our laboratory (Bowen and Balster, 1998; Bowen and McDonald, 2009).

2.3.2. Phase 2: repeated exposure

The mice were then repeatedly administered toluene at a concentration of 8000 ppm for 30 min/day for the next 14 consecutive days.

2.3.3. Phase 3: concentration–effect re-determination

Following the period of repeated toluene exposure, concentration-dependent effects of toluene on motor activity were redetermined ("Post" repeated exposure), and the development of tolerance and/or sensitization to toluene's effects was assessed, using the same procedure as in the initial determination, including the counterbalanced order of testing. During this "Post-chronic" phase of testing, the mice continued to receive chronic exposures to 8000 ppm toluene on the days between concentration–effect test days. Each mouse received only one exposure of toluene per day. (The Swiss Webster

strain was not re-tested with the 10,000-ppm toluene concentration after chronic toluene because of a technical error.)

2.4. Locomotor activity testing

Spontaneous locomotor activity was measured within the static exposure chamber via 3 sets of 16-beam infrared (I/R) emitter-detector arrays (Med Associates, St. Albans, VT) mounted on Plexiglas bases around the sides of the exposure chambers. Interruptions of I/R beams resulted in an analog signal being recorded by automated activity software (Open Field Activity Software [SOF-811], Med Associates, St. Albans, VT). This system quantified total beam breaks in both the vertical and horizontal planes, specifically encoding measures of distance traveled (cm; calculated from number of breaks of adjacent beams), ambulatory time (s), and number of rears. This automated measure of activity was transformed into 3-min Blocks over the duration of the session.

Mice were placed individually into the same exposure chamber in the same sequence each day. Activity was monitored once daily (Monday–Friday) for 30 min for five days prior to toluene exposures. This resulted in stable day to day levels of activity which served as a baseline against which toluene effects could be determined. The same animals were used for all subsequent testing.

2.5. Statistical analysis

Spontaneous locomotor activity as distance traveled during each 30-min toluene-exposure session was analyzed using $4\times5\times10\times2$ four-way, repeated-measures analysis of variance (ANOVA) with Strain (Balb/cByj, DBA/2J, C57BL/6J or Swiss Webster) as the between-subjects factor, and toluene Treatment (0 ppm, 100 ppm, 2000 ppm, 8000 ppm and 10,000 ppm), the 3-min time Blocks, and treatment times before and after repeated exposure (Pre vs. Post) as the nested within-subjects factors. Baseline activity levels were determined by averaging motor activity on three control air-only test sessions for each animal prior to the determination of the acute toluene concentration–effect curves. When comparing initial and redetermined effects on motor activity at each concentration, any changes in sensitivity would be seen as changes in the magnitude of the effects. Sensitization would be observed as a shift upwards or to the left in the concentration–effect curve while tolerance would be observed as a shift to the right. An alpha level of $p<0.05$ determined statistical significance. Tukey's *B post hoc* contrasts and simple main effects analyses were used to determine the locus of significant main effects and interactions.

2.6. Chemicals

The test chemical was toluene which was purchased from Fisher Scientific (T-324, Fisher Scientific Co., Fairlawn, NJ). Toluene was drawn directly from the bottle into a glass syringe and injected into the static vapor exposure system.

3. Results

3.1. Baseline distance traveled

The mean ($\pm$SEM) counts for the air-only baseline sessions before solvent testing for the Balb/cByj, C57BL/6J, DBA/2J and Swiss Webster strains were 831.8 ± 91.1, 1310.9 ± 238.3, 689.8 ± 67.9 and 453.3 ± 108.8, respectively. Significant baseline differences were observed across Strain ($F(3,38)=17.09$, $p<0.0001$) with *post hoc* analyses indicating that C57BL/6J mice were more active compared to all of the other strains ($p<0.05$). Fig. 1 shows the results of testing during the concentration–effect determinations assessed Pre-chronic toluene exposures.

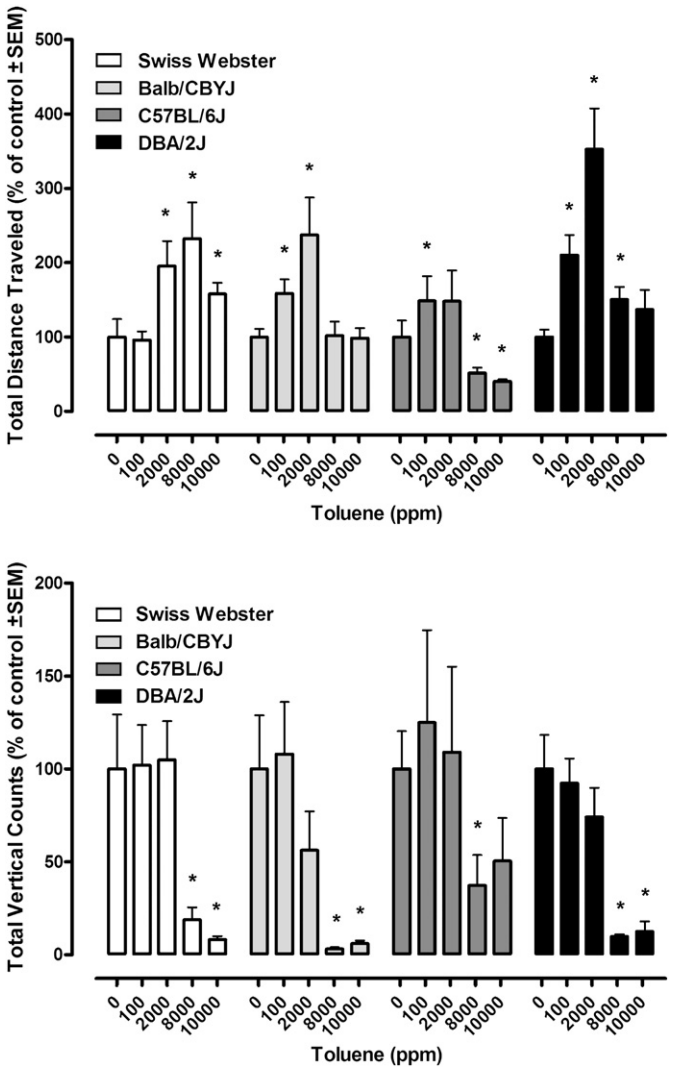

Fig. 1. Effects of inhaled 0, 100, 2000, 8000, or 10,000 toluene exposure on mouse distance traveled (top panel) and vertical counts (bottom panel). Mean total distance (top panel; % of air control $\pm$ SEM) and vertical activity (% of air control $\pm$ SEM) are as a sum of 3-min bins/session for 30-min exposures. *Significantly different from 0 ppm ($p\leq0.05$). ($N=11$ mice per concentration except C57BL/6J where $N=9$).

3.2. Acute toluene exposure

3.2.1. Distance traveled

The primary data of interest are the comparisons of toluene concentration–effect curves determined across the four strains of mice. There were significant main effects for Strain ($F(3,38)=4.02$, $p<0.05$) and toluene Treatment ($F(4,152)=18.67$, $p<0.0001$). As seen in the top panel of Fig. 1, the C57BL/6J strain was less active overall than the DBA/2J strain across all toluene concentrations examined ($p<0.05$). Initial acute toluene Treatment produced concentration-dependent, biphasic increases in locomotor activity in all four strains of mice (Fig. 1, top panel).

There was also a significant Strain $\times$ toluene Treatment interaction ($F(12,152)=4.59$, $p<0.0001$). While all strains showed biphasic concentration curves, *post hoc* analyses indicated that the Swiss Webster group was less active at the lowest toluene concentration (100-ppm) compared to the other strains at the same concentration ($p<0.05$; see Fig. 1, top panel). Overall, toluene concentrations up to 2000-ppm significantly increased locomotor activity when compared to the appropriate air-only control session, except the 100-ppm dose in the Swiss Webster group (Fig. 1, left side). The 2000-ppm

concentration of toluene produced significantly higher locomotor activity than 0-ppm air-exposed controls in all four strains of mice ($p<0.05$) and the DBA/2J mice were more active at this concentration than the C57BL/6J strain ($p<0.05$). With acute administration of ≥8000 ppm, locomotor activity was significantly increased above air control levels in the Swiss Webster and DBA/2J strains while no significant increases were observed in the Balb/cByj mice. Conversely, only significant decreases in activity were observed for these same toluene concentrations in the C57BL/6Js as compared to their air control levels.

A significant main effect was also observed for time Block ($F(9,342)=10.00$, $p<0.0001$), with locomotor distance traveled decreasing significantly as the session progressed (Fig. 2). There was a significant toluene Treatment × Block interaction ($F(36,1368)=17.69$, $p<0.0001$). At the two highest concentrations, significant decreases were observed in all four strains ~12 min into the session, perhaps indicating the beginning of a temporal biphasic effect of toluene. There was also a clear and significant Strain × toluene Treatment × Block interaction ($F(108,1368)=3.53$, $p<0.0001$). With the exception of the C57BL/6J strain and the 100-ppm concentration in the Swiss Webster group, every toluene concentration tested significantly increased locomotor activity initially when compared to the appropriate air-only control session with greater increases observed in the DBA/2J and Swiss Webster strains (Fig. 2, upper left and lower right panels). Finally, with the exception of the 8000-ppm concentration in the Swiss Webster strain, the two highest concentrations significantly decreased activity ~12 to 15 min into the session (as compared to the air-only control sessions) for all four strains of mice.

3.2.2. Vertical counts

There was a significant main effect involving toluene Treatment ($F(4,152)=19.99$, $p<0.0001$) on rearing movement (i.e., vertical counts). The Strain × toluene Treatment interaction was not significant ($p>0.96$; see Fig. 1, bottom panel). As with the other measure, a significant main effect was also observed for time Block ($F(9,342)=11.44$, $p<0.0001$), with total numbers of rears decreasing significantly as the session progressed (data not shown). There was a significant toluene Treatment × Block interaction ($F(36,1368)=3.11$, $p<0.0001$). At the two highest concentrations, significant decreases were observed in all four strains ~12 min into the session, perhaps indicating the beginning of a temporal biphasic effect of toluene. There was also a significant Strain × toluene Treatment × Block interaction ($F(108,1368)=1.78$, $p<0.0001$) with the 2000-ppm concentration decreasing vertical activity ~6 to 9 min into the session (as compared to the air-only control sessions) for the Balb/cByj mice which was not observed in the other strains (data not shown).

3.3. Repeated toluene exposure (Pre/Post examination)

For the comparisons of concentration–effect curves determined after repeated toluene exposures, the 10,000-ppm toluene concentration was not included in the overall Pre/Post analysis because it was not tested in the Swiss Webster strain.

3.3.1. Distance traveled

The ANOVA demonstrated a significant main effect of Pre/Post ($F(1,38)=36.88$, $p<0.001$) and, as seen in each of the four top panels

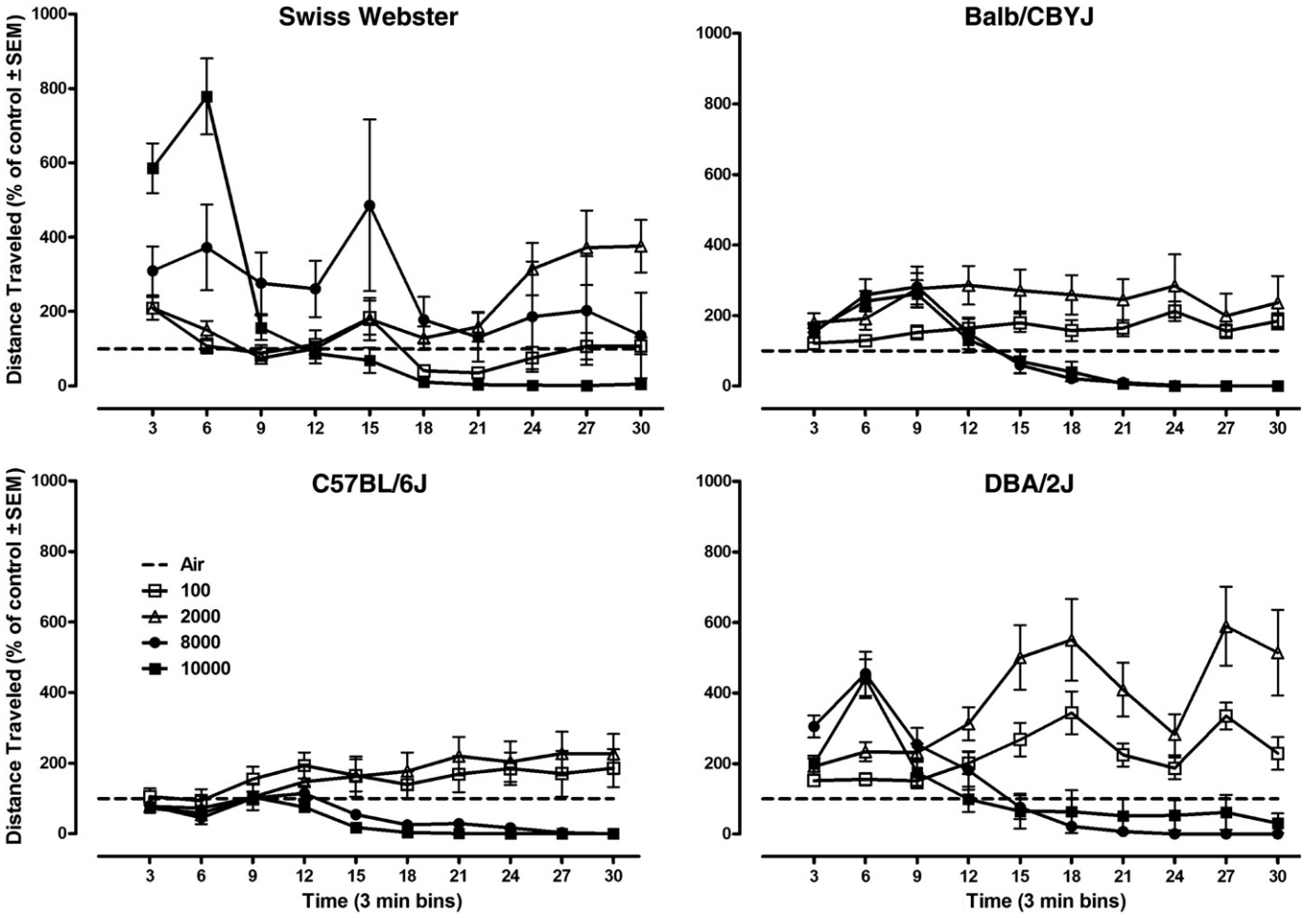

Fig. 2. Concentration- and time–effect curves for inhaled toluene on mouse distance traveled (% of air control ± SEM). Activity for the four strains of mice is shown in ten consecutive 3-min segments for the 30-min exposures.

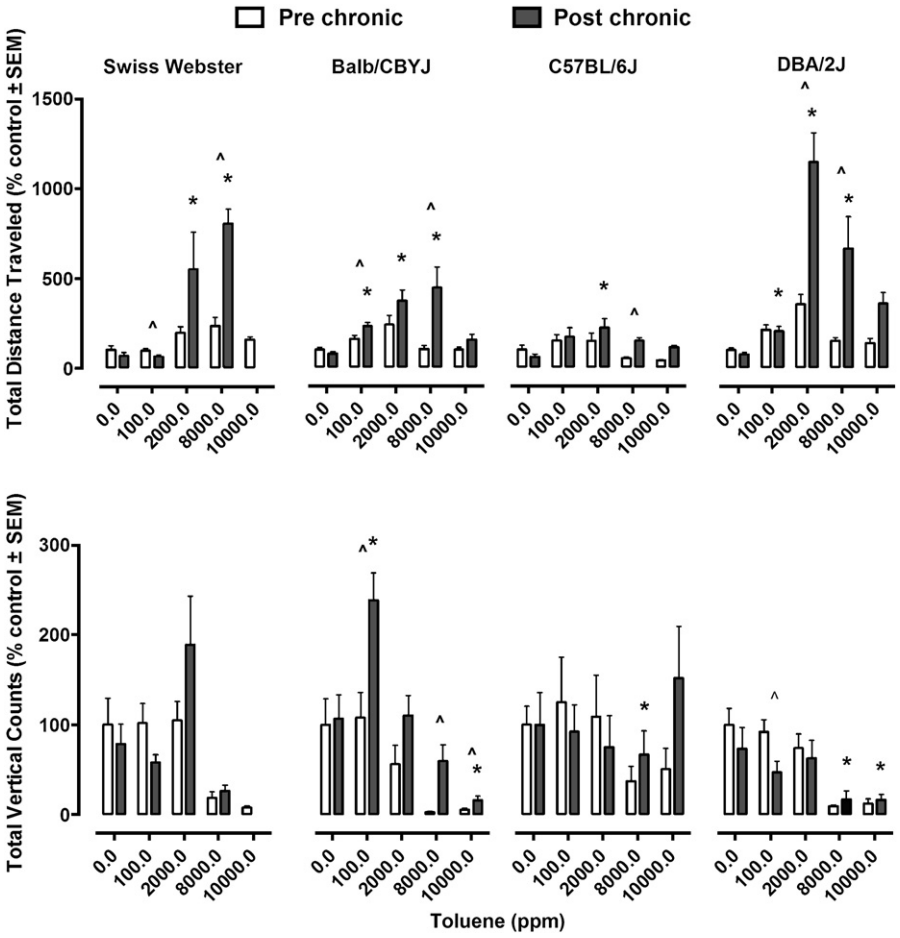

Fig. 3. Effects of inhaled 0, 100, 2000, 8000, or 10,000 toluene exposure on mouse distance traveled (top panel) and vertical counts (bottom panel) over a period of repeated 8000 ppm toluene exposures when it was administered before (empty bar) or after (filled bar). Mean total distance (top panel; % of air control ± SEM) and vertical activity (% of air control ± SEM) are as a sum of 3-min bins/session for 30-min exposures. *Significantly different from Post 0 ppm ($p \leq 0.05$). ^Significant Pre/Post differences ($p \leq 0.05$). ($N = 11$ mice per concentration except C57BL/6J where $N = 9$).

of Fig. 3, significant sensitization was observed in all four strains when the challenge concentrations of toluene used in the initial acute tests were re-tested after the 14-day repeated exposure regimen. (To facilitate direct comparisons between the "Pre-" and "Post-chronic" phases, the open bars for the "Pre-chronic" phase repeat the data presented in Fig. 1). Separate assessments revealed Pre/Post differences at the 100 and 8000 concentrations in the Balb/cByj and the Swiss Webster strain, as well as differences in the 2000 and 8000 concentrations in the DBA/2J and the 8000 concentration in the C57BL/6J strain. Activity levels after the same toluene exposures were significantly higher in every case except the 100-ppm concentration in the Swiss Webster strain where activity levels were lower, suggestive of tolerance at doses ≥100 ppm. In addition, there was a significant interaction between mouse Strain and Pre/Post treatment, ($F(3,38) = 4.97$, $p < 0.01$) with larger increases in activity from pre- to post-testing seen in the DBA/2J strain than in any of the other strains receiving repeated toluene exposures. The ANOVA also revealed a significant interaction of toluene Treatment concentration with the Pre/Post condition, ($F(3,114) = 9.74$, $p < 0.001$), which is shown in Fig. 3 as a greater increase in activity from Pre to Post seen with higher toluene concentrations (i.e., 2000 and 8000 ppm). Finally, ANOVA revealed a significant 3-way interaction of toluene Treatment × Pre/Post condition × Strain, ($F(9,114) = 1.99$, $p < 0.05$), which results from the DBA/2J strain showing greater increases in locomotor activity from Pre to Post at the 2000-ppm test concentration than the other strains and the decrease in locomotor activity in the Swiss Webster strain from Pre to Post at the 100-ppm concentration.

The time course for toluene effects after the development of sensitization due to chronic exposure to 8000 ppm toluene is shown in Fig. 4. A significant main effect of time ($F(9,342) = 8.06$, $p < 0.001$), a time × Strain interaction, ($F(27,342) = 12.06$, $p < 0.001$), and a time × Strain × toluene Treatment interaction, ($F(81,1026) = 5.80$, $p < 0.001$ were observed when the challenge concentrations of toluene used in the initial acute tests were re-tested after the 14-day repeated exposure regimen. The lowest concentration of toluene (100 ppm) resulted in relatively stable activity locomotion over the 30-min session while the higher concentration of 2000 ppm resulted in greater activity-increasing effects across the session and more so in the DBA/2J mice. The highest concentrations of 8000 ppm and 10,000 ppm resulted in the greatest activity-increasing effects early in the exposure in all but the C57BL/6J strain with lower activity counts over the last 20 min of exposure.

3.3.2. Vertical counts

As seen in each of the four bottom panels of Fig. 3, the ANOVA demonstrated that no significant main effect was observed for Pre/Post ($p > 0.74$) or Strain ($p > 0.10$). There was however a significant Strain × Pre/Post interaction, ($F(3,38) = 5.29$, $p < 0.01$) with changes in vertical counts from pre- to post-testing seen in the Balb/cByj and DBA/2J strains than in the Swiss Webster strain. While there was no significant toluene Treatment × Pre/Post interaction, ($p = 0.07$), there was a significant 3-way toluene Treatment × Pre/Post × Strain interaction, ($F(9,114) = 2.29$, $p < 0.05$) with significant increases in vertical counts for 100-ppm toluene from pre- to post-testing seen in the Balb/cByj strain.

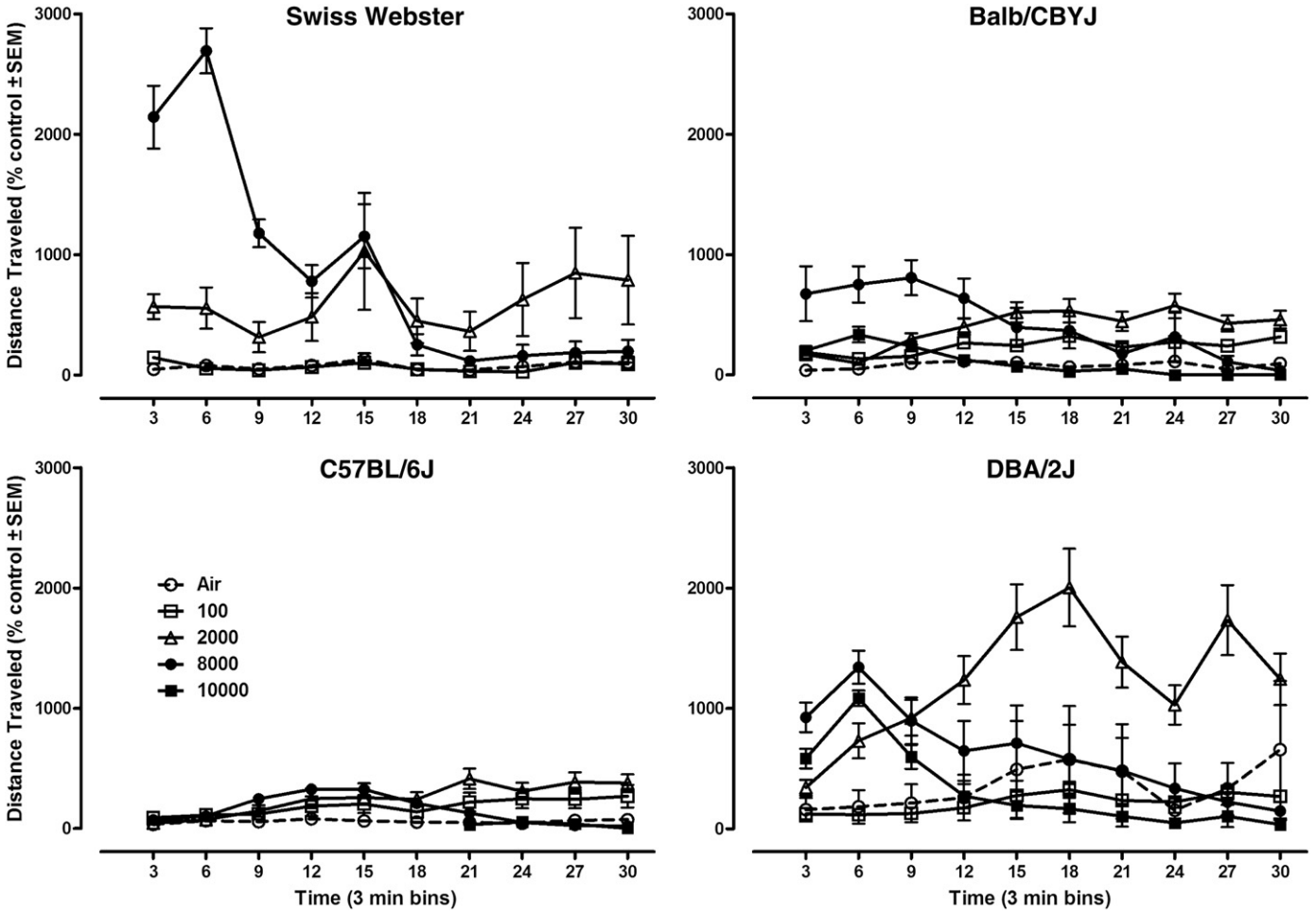

Fig. 4. Concentration- and time-effect curves for inhaled toluene on mouse distance traveled (% of air control ± SEM) after 15 day repeated 8000 ppm toluene exposure. Activity for the four strains of mice is shown in ten consecutive 3-min segments for the 30-min exposures.

A significant main effect of time ($F(9,342) = 17.04$, $p < 0.001$), a time × Strain interaction, ($F(27,342) = 4.01$, $p < 0.001$), and a time × Strain × toluene Treatment interaction, ($F(81,1026) = 2.16$, $p < 0.001$ was observed when the challenge concentrations of toluene used in the initial acute tests were re-tested after the 14-day repeated exposure regimen. While the lowest concentration of toluene (100 ppm) resulted in increases in vertical activity over the 30-min session for only the Balb/cByj strain, the higher concentration of 2000 ppm produced greater increases in vertical activity at the beginning of the session and more so in the Swiss Webster and Balb/cByj mice (data not shown). The still higher concentration of 8000 ppm resulted in initial increases to total vertical activity for Balb/cByj mice followed by no vertical activity ~12 to 15 min after exposure, a pattern that was not observed for any other strain (data not shown).

4. Discussion

The primary purpose of the present research was to characterize differences among four strains of mice in locomotor activity following acute and repeated exposures to toluene. These studies employed an inhalation exposure system which mimicked inhalant abuse levels of exposure and expand previous inhalant investigations by showing that locomotor activity is altered when exposed to graded increases in toluene concentration. Further, these toluene-induced increases were dependent on mouse strain.

Three inbred strains (Balb/cByj, DBA/2J and C57BL/6J) and one outbred strain (Swiss Webster) of mice were used. These strains were chosen because they are the most commonly used and genetically divergent of the inbred strains and have well-characterized differences in their responses to other drugs of abuse, especially ethanol (Crabbe et al., 1994; Crabbe et al., 2005; Phillips, 1997). Differences in ethanol responses seemed particularly relevant to toluene because both are organic solvents and previous studies showed that both can have CNS depressant effects (see reviews (Bowen et al., 2006; Evans and Balster, 1991). The Swiss Webster strain was included because it represents a genetically heterogeneous stock of mice and it has been the strain most frequently used in earlier reports examining the behavioral effects of solvent exposure (Bowen et al., 2006; Cruz and Bowen, 2008; Evans and Balster, 1991). The current acute and repeated toluene exposure procedure has been used previously to study the effects of other abused drugs, so a second goal was to compare the results obtained with toluene in these selected strains of mice to those reported previously for other drugs of abuse. It may be possible to obtain some information from animal studies relevant to predicting the abuse potential of solvents by determining the degree of overlap in their CNS effects with those of known drugs of abuse. To our knowledge, this represents the first report of the acute and repeated behavioral effects of toluene using these strains of mice.

Interpretation of stain differences in toluene responsivity must account for the significant baseline differences in horizontal and vertical activities evident across these mouse strains. The C57BL/6J strain was significantly more active than the three other strains. As stated in the Results section, the mean count for the air-only baseline sessions for total distance traveled for the C57BL/6J was ~1310 cm which was more than double than the levels observed for either the DBA/2J or Swiss

Webster mice. This substantially greater baseline activity was not limited to horizontal locomotion but was also observed for vertical activity (rearing) as well (data not shown). These marked differences in levels of horizontal and vertical activities for the C57BL/6J mice have been demonstrated previously using a number of behavioral tests (Cabib et al., 1990, 2002; Puglisi-Allegra and Cabib, 1997). For example, when activity levels of C57BL/6J mice were compared to DBA/2J mice using an open-field paradigm, C57BL/6J mice show significantly more horizontal and vertical locomotions than DBA/2J mice (Cabib et al., 1990, 2002; Puglisi-Allegra and Cabib, 1997).

A current key finding was that acute toluene administration resulted in qualitatively similar acute behavioral effects in all four strains of mice (see Fig. 1). That is, all strains clearly demonstrated toluene inhalation exposure-produced biphasic effects on spontaneous locomotor activity in mice. At the lowest effective concentrations (100–2000 ppm), toluene increased locomotor activity monophasically with time, and this increased activity was sustained throughout the exposure in all four strains of mice (see Fig. 2). At higher concentrations (8000–10,000 ppm), locomotor activity was decreased for all strains (with the exception of the 8000-ppm concentration in the Swiss Webster strain). The time course of this locomotor activity revealed a biphasic action that was initially increased above baseline (with the exception of the C57BL/6J mice) and began to decrease as the test session progressed. Thus, the biphasic action of toluene was demonstrated both by concentration- and time-dependent changes in behavior. In addition, the results show that acute toluene induced a significantly higher maximal locomotor stimulant effect in the inbred DBA/2J and the outbred Swiss Webster mice when compared to the C57BL/6J and Balb/cByj mice which had minimal increases in locomotor activity (see Figs. 1 and 2). Conversely, the results also demonstrate that as compared to baseline activity levels, the highest acute toluene concentrations produced the greatest reduction in measures of locomotor activity in the C57BL/6J as compared to the other strains tested (see top panel of Fig. 1). It is also interesting to note that the 100-ppm concentration of toluene produced sustained motor activity-increasing effects throughout the sessions in the inbred strains while relatively minimal increases were observed in the outbred Swiss Webster strain.

There was also a clear effect of repeated, "chronic" toluene exposure, in that sensitization was evident with locomotor activity, where the overall magnitude of the motor activation produced by toluene increased with repeated exposure. Re-testing of acute toluene exposures after repeated administration demonstrated that toluene continued to produce dose-dependent effects on spontaneous locomotor activity but at levels significantly higher than were observed initially. As was observed with acute exposure, the repeated toluene dosing resulted in strain differences. Significantly higher maximal locomotor stimulant effects were documented in the DBA/2J mice, most notably at the 2000-ppm concentration, as compared to the Balb/cByj and Swiss Webster mice while the C57BL/6J mice had only minimal increases in locomotor activity (Fig. 3). Examination of the time-course data shows that toluene increased locomotor activity monophasically with time and this increased activity was sustained throughout the exposure in all four strains of mice (Fig. 4). The two highest concentrations of 8000 and 10,000 ppm toluene continued to produce biphasic results in all but the C57BL/6J mice. It should also be pointed out that the choice of 8000 ppm toluene for repeated administration may have also played a major role in the observed differences in post-chronic effects, especially since this concentration of toluene originally resulted in different acute effects across strains. It is possible that lower or higher concentrations of repeatedly administered toluene could result in very different outcomes.

To our knowledge, these findings are the first to report strain differences in toluene effects on locomotor activity. However, the current results are similar to previous reports which have demonstrated that acute inhalation of toluene in Swiss Webster mice produces a profile of effects that progress from motor excitation at lower concentrations (i.e., 500–4000 ppm) to motor impairment, sedation and anesthesia at concentrations above 6000 ppm (Bowen and Balster, 1998). Similar increases in motor activity have been found when toluene was administered in rats via inhalation (Himnan, 1984) and systemically (Riegel et al., 2003; Riegel and French, 1999). The differences in sensitivity to toluene's effects in the present investigation have been reported for other pharmacological agents comparing these strains. In particular, C57 mice have been shown to be more sensitive than DBA to the behaviorally activating effects of direct and indirect dopamine agonists (Puglisi-Allegra and Cabib, 1997). For example, Anisman et al. demonstrated that increases in locomotor activity produced by amphetamine were much more pronounced in the C57 strain than in the DBA strain of mice (Anisman and Cygan, 1975; Anisman et al., 1975). Others have reported that lower doses of cocaine are required to reduce operant responding for food in Balb/cByJ and C57BL/6J whereas higher doses were required in DBA/2J mice (Deroche et al., 1997; Heyser et al., 1997). The current results are also comparable to previous reports that C57 mice are less sensitive when compared with DBA mice to the sedative effects of ethanol (Crabbe, 1983; Crabbe et al., 1994, 1982; Escher and Mittleman, 2004; Phillips et al., 1994).

These effects of toluene on locomotor activity are consistent with previous reports of the development of sensitization after repeated exposure to toluene (Hinman, 1987; Wiaderna and Tomas, 2000, 2002). Sensitization to the locomotor activation produced by drugs of abuse is well established and often used as a model for studying the development of dependence and risk for abuse (Kalivas and Stewart, 1991; Kuczenski and Segal, 2001; McDougall et al., 1999). Many studies point to a role of dopaminergic activation in the development of sensitization to stimulants and opiates (Pierce and Kalivas, 1997; Robinson and Berridge, 1993). There is evidence that dopaminergic systems play a role in the acute effects of toluene on locomotor activity as well (Riegel et al., 2003; Riegel and French, 1999, 2002). Indeed, cross-sensitization has been shown from cocaine to toluene (Beyer et al., 2001). One other possible explanation for the increases in activity is the possibility of tolerance to the activity inhibiting effects of higher doses of toluene. Evidence for this possibility can be found in the ethanol literature where the dose–response curve for ethanol-based increases in locomotion is seen as overlapping with the ethanol-based decreases in locomotion with repeated exposure increasing the ED 50 for locomotor inhibition (Erwin et al., 1992). Since the development of sensitization and tolerance are commonly seen with drugs of abuse and since this abused solvent produces this phenomenon as well, tests for sensitization and/or tolerance may be useful as part of a battery for the abuse potential assessment of inhalants (Balster, 1987).

While the exact mechanisms for these different responses to toluene are unclear, there is considerable research documenting the pharmacological, physiological, and behavioral differences between these strains. While an extensive review of this literature is not feasible here, several excellent reviews do exist (Cabib et al., 2002; Puglisi-Allegra and Cabib, 1997). Among these differences are some that may be pertinent for beginning to understand the neurobehavioral responses to toluene exposure in these inbred mouse strains. For example, C57BL/6 and DBA/2 mice have been shown to vary in several parameters of the dopamine system which are associated with behavioral changes. Cabib et al. (1998) have demonstrated that C57BL/6 mice have very low densities of D2 dopamine receptors in the ventral tegmental area as compared to DBA/2 mice. These same authors have also reported that C57BL/6 mice have increased levels of D2 postsynaptic receptors within the nucleus accumbens as compared to DBA/2 mice. These differences in pre and postsynaptic populations have also been postulated to result in enhanced dopamine transmission within the mesoaccumbens dopamine system in C57BL/6 mice and may explain the "hyperactive response" that these mice have to novel and conflicting situations (Cabib et al., 1990, 1998, 2002; Puglisi-Allegra and Cabib, 1997). Pharmacological investigations have also shown that the C57 are much more sensitive than DBAs to the stimulant effects of amphetamine and apomorphine (Cabib et al.,

1998; Puglisi-Allegra and Cabib, 1997). Strain-dependent effects of stress have also been demonstrated in ethanol consumption with chronic swim stress producing significant decrease in ethanol consumption in DBA/2J and BALB/cByJ, but not C57BL/6J mice (Boyce-Rustay et al., 2007, 2008).

In summary, similar to what is observed with many drugs of abuse, repeated exposure to toluene resulted in both tolerance and sensitization to its behavioral effects. The major determinant of whether tolerance or sensitization occurred was the concentration of toluene acutely tested. In addition, this study presents evidence that another major determinant was the strain of mouse that was examined with differential responses of these four strains regarding locomotor activity increases or decreases following toluene exposure. Because all of the behavioral testing was done under exactly the same exposure conditions for each of the four strains, variations in exposure test conditions could not have been the major variable for the differences that were observed. These data support the hypothesis that these behavioral effects to toluene exposure are genotype dependent and provide a preliminary basis for the biological mechanisms responsible for these differences.

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