Psychopharmacology (2025) 242:1335–1349
https://doi.org/10.1007/s00213-024-06731-5

ORIGINAL INVESTIGATION

Repeated toluene inhalation in male and female adolescent rats induces persistent drug preference and impairs cognitive and social behavior

Joannes Luke B. Asis[1,2] · Ajina C. Carampel[1,2] · Jariel Naomi B. Bacar[2] · Johanna C. Munar[2] · Cynthia Grace C. Gregorio[3] · Paul Mark B. Medina[4] · Leslie Michelle M. Dalmacio[4] · Jesus Emmanuel A. D. Sevilleja[5] · Gregory J. Quirk[2] · Rohani Cena-Navarro[2]

Received: 1 October 2024 / Accepted: 2 December 2024 / Published online: 18 December 2024
© The Author(s) 2024

Abstract

Rationale Adolescent inhalant use is an understudied and undertreated disorder, particularly in females. Chronic exposure to inhalants, like toluene, can have long-lasting effects on behavior. However, most animal studies lack the incorporation of both sexes and do not focus on the abstinence period.

Objective We assessed the behavioral effects during prolonged abstinence following repeated toluene inhalation in adolescent male and female rats.

Methods We repeatedly exposed adolescent male and female Sprague Dawley rats to toluene vapor (1500 or 3000 ppm) for 6 days using the conditioned place preference (CPP) procedure. We tested drug-associated context preference, locomotion, anxiety-like behavior, object memory, social preference, and cognitive flexibility across 22 days of abstinence.

Results In females, 3000 ppm toluene increased CPP on days 8 and 22 of abstinence but this effect did not reach significance in males. Instead, males showed a significant increase in locomotion on days 7 and 21. Toluene also impaired social novelty preference and reversal learning during long-term abstinence, but not anxiety-like behavior or object recognition memory.

Conclusions Our rodent findings suggest that female inhalant users may show persistent drug preference during abstinence following chronic use. Furthermore, prolonged cognitive and social deficits should be addressed in treatment programs for adolescents.

Keywords Inhalants · Addiction · Abstinence · Conditioned place preference · Reversal · Social novelty

Joannes Luke B. Asis and Ajina C. Carampel are equal contributors to this work and designated as co-first authors.

✉ Rohani Cena-Navarro
rbcena@up.edu.ph

1 College of Medicine, University of the Philippines Manila, Manila, Philippines

2 National Institute of Molecular Biology and Biotechnology, National Institutes of Health, University of the Philippines Manila, 623 Pedro Gil Street, Ermita, 1000 Manila, Philippines

3 Institute of Chemistry, College of Science, University of the Philippines Diliman, Quezon City, Philippines

4 Department of Biochemistry and Molecular Biology, College of Medicine, University of the Philippines Manila, Manila, Philippines

5 Mental Health Research Unit, Office for Special Concerns, National Center for Mental Health, Mandaluyong, Philippines

Introduction

Inhalant use disorder (IUD) is a significant health problem worldwide that is prevalent among male and female adolescents (Nguyen et al. 2016). During the COVID-19 pandemic, inhalant use in adolescents increased and remained elevated (Pelham et al. 2023). While some countries report a higher prevalence of IUD in males, others report a higher prevalence in females (ESPAD Group 2020; Crossin and Arunogiri 2020). Treating IUD remains a challenge and can be hampered by long-lasting impairments in social and cognitive functions associated with chronic use (Howard et al. 2010; Woodward and Braunscheidel 2023). Animal studies could be helpful in characterizing the long-term effects of toluene, the most commonly abused inhalant (Cruz et al. 2014). However, most prior studies did not characterize the effects of prolonged abstinence following repeated toluene

exposure, and assessed only males (reviewed in Cruz & Bowen 2021).

A commonly used animal model of IUD is conditioned place preference (CPP) with inhaled toluene vapor. Repeated exposure to toluene vapor (700–5000 ppm for 6 conditioning sessions) has been shown to increase CPP in adolescent male rodents (Gerasimov et al. 2003; Schiffer et al. 2006; Lee et al. 2006; Wayman and Woodward 2018) for up to 7 days, but not 30 days, into abstinence (Wayman and Woodward 2018). Toluene has also been found to impair object memory in adolescents for several weeks into abstinence (Lin et al. 2010; Montes et al. 2017), but had no lasting effect on anxiety-like behavior (Lin et al. 2010; Bowen et al. 2018). Repeated exposure to toluene has also been shown to decrease social interaction in adolescent male mice (Lin et al. 2010). However, toluene's effects on preference for social novelty has never been tested in adolescents, nor its effects on most cognitive behaviors.

In our study, we used an adolescent rat model of toluene CPP to assess the behavioral effects of repeated toluene inhalation at moderate exposure concentrations (1500 and 3000 ppm). We assessed behavior during 22 days of abstinence in both males and females. We examined drug-associated context preference, locomotion, anxiety-like behavior, object memory, social preference, and cognitive flexibility. Our aim was to carry out a comprehensive assessment of the behavioral effects of repeated toluene exposure in both sexes that could be relevant for designing treatment programs.

Methods

Animals

Male ($n = 96$) and female ($n = 99$) adolescent Sprague Dawley (SD) rats at 21 days of age (P21) were purchased from BioLASCO (Taiwan). Rats of the same sex were pair-housed in polypropylene cages (425 × 266 × 185 mm) with corn cob beddings and given ad libitum access to water and feeds (Altromin 1324, Germany). The animal facility was a temperature- and humidity-controlled environment (20–22 °C, 40–70% humidity) with a 12-h reversed light–dark cycle (lights on: 1900–0700). Experimental procedures started in adolescent (P29) rats after 7 days of acclimatization and under the dark phase. All experiments were approved by the University of the Philippines Manila Institutional Animal Care and Use Committee (protocol no. 2021–008).

Conditioned place preference

The toluene conditioned place preference (CPP) protocol was adapted from previous studies in male SD rats (Gerasimov et al. 2003; Lee et al. 2004, 2006; Wayman and Woodward 2018) with some modifications.

The CPP apparatus (La Jolla Alcohol Research, Inc. (LJARI), USA) consisted of three distinct chambers: a smaller gray middle chamber with a smooth acrylic floor (21 × 13 × 21 cm), a white conditioning chamber with a smooth rubber floor (21 × 27 × 21 cm), and a black conditioning chamber with a textured rubber floor (21 × 27 × 21 cm). Manually operated guillotine doors separated the chambers. The black chamber where toluene was given had a gasket installed around the lid and an inlet for air or toluene vapor was installed at the top. The white chamber had holes at the lid to let air in. All three chambers had exhaust tubes installed. Liquid toluene (99.5% analytical grade, RCI Labscan, Thailand) was vaporized using custom-made bubblers attached to computer-controlled air flow regulators (LJARI, USA) at 2 or 3 lpm (for 1500 or 3000 ppm toluene vapor output, respectively). Toluene vapor concentrations within the black chamber were calibrated to averages of 1500 ppm (range: 1000 to 1900 ppm) or 3000 ppm (range: 2200 to 3600 ppm) using a portable toluene gas detector (Cosmos XP-3360II, DOD Technologies, USA) with measurements recorded every 15 s. Once rats were placed inside the chamber, there was an initial 5-min period to allow toluene vapor concentrations to reach target levels (1500 or 3000 ppm) which was then maintained for 30 min (Supplementary Fig. 1).

We chose 1500 and 3000 ppm to determine if there is a relationship between the toluene exposure concentration and behavioral effects. Toluene CPP has been previously demonstrated using a concentration of as low as 700 ppm in mice (Funada et al. 2002) and 1895 ppm in rats (Gerasimov et al. 2003), hence we explored whether 1500 ppm will produce CPP in SD rats. We chose 3000 ppm as the higher concentration based on previous studies demonstrating CPP at this level (Gerasimov et al. 2003; Lee et al. 2006; Wayman and Woodward 2018). We did not include 5000 ppm in our study based on prior reports that this concentration failed to produce CPP, and even triggered conditioned place aversion (Schiffer et al. 2006; Lee et al. 2006). In a pilot study in which we exposed rats to 5000 ppm toluene vapor, we observed lethargy and excessive salivation, which could interfere with learning drug-context associations during CPP conditioning.

The CPP protocol consisted of three phases: preconditioning, conditioning, and test phases. *Preconditioning phase:* Adolescent rats (P29 to P52) were placed in the center gray chamber with the guillotine doors closed. After 2 min, the doors were opened, and the rats were allowed to explore all three chambers for 15 min. Each rat's initial preference score was calculated by the time spent in the black chamber (where toluene will be given) over the sum of the time spent in both black and white conditioning chambers.

Rats were assigned to match the average group preference scores between air (control group), 1500 ppm toluene, or 3000 ppm toluene groups. *Conditioning phase:* Toluene groups were exposed to toluene vapor in the black chamber and air in the white chamber with the guillotine doors closed (Fig. 1A). The control group was exposed to air in both conditioning chambers. For 12 days, rats underwent one 30-min conditioning session per day. Rats received air on the first day, then air (control group) or toluene vapor (toluene groups) the next day, with alternating air and toluene conditioning sessions on subsequent days. The toluene groups received a total of six air and six toluene conditioning sessions, while the control group received 12 air conditioning sessions (Fig. 1B). *Test phase:* One day after the last toluene conditioning day (day 13, termed test day 1 (D1), rats were placed in the center gray chamber with the guillotine doors closed. After 2 min, the doors were opened, and the rats were allowed to explore all three chambers for 15 min. A CPP ratio score was calculated as the time spent in the black chamber (where toluene was given) divided by the sum of the time spent in both black and white chambers (Yates et al. 2013; Sun et al. 2017; Jia et al. 2023). A CPP difference score was calculated as the time spent in the black chamber minus the time spent in the white chamber during test. The CPP test was repeated on D8 and D22 of test phase.

Experimental design

To determine the behavioral effects of repeated toluene exposure during the test phase, a total of 87 male and 90 female adolescent SD rats were exposed to air, 1500 ppm toluene, or 3000 ppm toluene. These rats were then tested for various behaviors during the 22-d test phase (Fig. 1B). Due to a large number of behavioral tests, we did not run all tests in each rat. Prior to all behavioral tests, rats were allowed to habituate to the testing room for 1 h. Behavior was recorded using video cameras and analyzed using ANY-Maze version 7.33 (Stoelting Co., USA).

To determine the concentration of toluene in the blood following inhalation, 9 male and 9 female rats were exposed to air or 3000 ppm toluene vapor under the CPP conditioning schedule (Fig. 1B). Blood was drawn from the tail vein immediately after the first and last toluene vapor exposure and 24 h after the last exposure. Toluene levels from the blood samples were measured using a validated headspace gas chromatography method.

Elevated plus maze test

Rats underwent the elevated plus maze (EPM) test on D2, D6, and D20 of test phase. The EPM (Stoelting Co., USA)

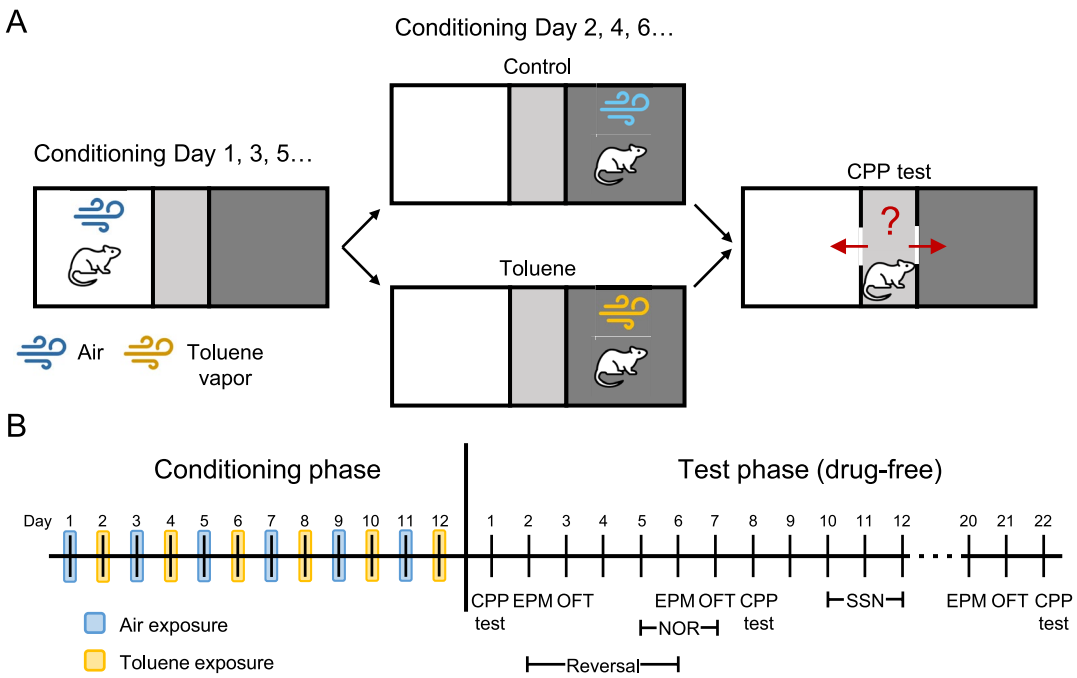

Fig. 1 Experimental design. **A** Toluene CPP conditioning was performed using a three-chambered apparatus in which rats were exposed to air in a white chamber and to toluene vapor in a black chamber. Control rats were exposed to air in both chambers. During test, rats were tested for chamber preference. **B** The conditioning phase consisted of 12 days of alternating air and toluene exposures. One day after the last toluene exposure, rats initiated 22 days of drug-free abstinence and underwent a battery of behavioral tests. *CPP* Conditioned Place Preference, *EPM* Elevated Plus Maze, *OFT* Open Field Test, *NOR* Novel Object Recognition, *SSN* Sociability and Social Novelty Tests, *Reversal* Reversal Learning

had two enclosed arms (50 × 10 cm) with 40 cm-high walls and two open arms without walls (50 × 10 cm). The maze was elevated 50 cm above the floor. Rats were placed in the center of the maze facing one of the open arms and allowed to explore freely for 5 min. The ratio of time spent on the open arms divided by the time spent in both open and closed arms was calculated. Two female rats were excluded from the D2 test because they fell from the apparatus.

Open field test

Rats underwent the open field test (OFT) on D3, D7, and D21 of test phase. The open field apparatus (Stoelting Co., USA) was a 100 × 100 cm box with 50 cm-high opaque walls and a smooth acrylic floor. Rats were placed in the center of the open field and allowed to explore freely for 10 min. Distance travelled and percentage of time spent in the center of the open field (50 × 50 cm, 25% of total area) were obtained.

Novel object recognition test

Rats underwent the novel object recognition (NOR) test on D5–D7 of test phase. The arena used for the NOR test was the open field apparatus (Stoelting, USA) divided into four quadrants by a wooden divider (height: 60 cm), which enabled rats to be tested four at a time. Each NOR arena measured 50 × 50 × 60 cm. The NOR test consisted of three phases: acclimatization, habituation, and testing phases. *Acclimatization*: On D5 of test phase, rats were placed in the empty arena for 5 min. *Habituation:* On D6 of test phase, the rats were presented with two identical objects in the arena for 10 min. We used circular glass jar lids (5.5 cm height × 11.5 cm diameter) in half of the rats and wooden spherical blocks (9 × 8 × 3.5 cm) in the other half. *NOR Test:* On D7 of test phase, rats were presented with one of the familiar objects from the previous day and a novel object (wooden block or glass lid) for 5 min. Exploration time for a given object was defined as the time spent sniffing or moving within 2 cm of the object. Time was measured manually with stopwatches, with observers blinded to the group and object novelty. The NOR score was calculated as the exploration time for the novel object divided by the sum of exploration times for both objects.

Sociability and social novelty test

Rats underwent the sociability and social novelty (SSN) test on D10–D12 of test phase. This task was based on a prior study that modeled social deficits in a mouse model of autism spectrum disorder (Moy et al. 2004). Several prior studies have adapted this task in rats to study social motivation and stress (Lukas et al. 2011; Netser et al. 2020; Potrebić et al. 2022; Shirenova et al. 2023), but not addiction. The social test apparatus (Ugo Basile, Italy) was a rectangular three-chamber testing apparatus with transparent acrylic walls, with each chamber measuring 40 × 40 × 40 cm. The middle chamber was separated from the left and right chambers by manually operated guillotine doors. Two identical cylindrical enclosures (25 cm height × 15 cm diameter) with metal railings and polyvinyl chloride roof and floor were placed in the middle of the left and right chambers. The SSN test consisted of three phases: habituation, sociability test, and social novelty test. *Habituation:* On D10 of test phase, test rats were initially placed in the closed center chamber for 2 min and were allowed to explore the three chambers and empty cylindrical enclosures for 10 min. *Sociability test:* On D11 of test phase, a demonstrator rat of the same sex and age as the test rat was placed in one of the cylindrical enclosures, while wooden blocks were placed in the other cylindrical enclosure. The chamber containing the demonstrator rat (left vs. right) was counterbalanced for all the groups. Each tested rat was given the opportunity to explore both side chambers for 10 min after being placed into the center chamber. Exploration time for a given enclosure was defined as the time spent sniffing or moving within 3 cm of the enclosure and excluded time when the center of the animal was on top of the enclosure. Exploration time was measured using ANY-Maze version 7.33 (Stoelting Co., USA). The sociability score was calculated as the exploration time for the cylindrical enclosure with the demonstrator rat divided by the sum of the exploration times for both cylindrical enclosures. *Social novelty test:* On D12 of test phase, demonstrator rats of the same sex and age as the test rat were placed in both cylindrical enclosures, one of which was the rat used during the sociability test (familiar rat) and the other rat was one that the test rat has not encountered prior to the test (novel rat). The position of the demonstrator rats was counterbalanced for all the groups. Each test rat was allowed to explore both enclosures for 10 min. The social novelty score was calculated as the exploration time for the cylindrical enclosure with the novel rat divided by the sum of the exploration times for both cylindrical enclosures.

Reversal learning

Appetitive reversal learning (adapted from Stephenson-Jones et al. 2016 with some modifications) was assessed in an operant conditioning apparatus with two bars (left and right) and a pellet feeder between the bars (Coulbourn Instruments, USA). The bars and feeder were controlled by ANY-Maze version 7.33 (Stoelting Co., USA). We performed reversal learning in a limited number of new animals that did not undergo CPP training and other behavioral tests. We chose to examine only the 3000 ppm concentration of toluene because the 1500 ppm concentration did not induce CPP. This subset of rats was food-restricted to maintain

90–95% of their free-feeding weight. The reversal learning experiment consisted of the following phases: initial training, toluene exposure, retraining, discrimination, reversal 1, reversal 2, and reversal 3, with each reversal of increasing difficulty. *Initial training:* Before toluene exposure, rats underwent bar press training for sucrose pellets for 10 days. Each press for either left or right bar was rewarded with one sucrose pellet (100% reinforcement). *Toluene exposure:* Rats were then exposed to 3000 ppm toluene vapor in the CPP apparatus for 30 min every other day across a 12-d period. During non-toluene exposure days, rats remained in their home cages. *Retraining:* On D1 of test phase, retraining for bar pressing was performed with 100% reinforcement. *Discrimination:* On D2–D3 of test phase, rats were trained to discriminate a bar that delivers pellets 3 out of 4 times (75% reinforcement) from a bar that never delivered pellets (0% reinforcement). Each session lasted 8 min. *Reversal 1, 2, and 3:* On D4 of test phase, for reversal 1, the positions of the 75%-bar and 0%-bar were reversed. On D5 of test phase, for reversal 2, the positions were reversed again, however, the reinforcement schedule of the 0%-bar was modified to 20% (2 out of every 5 presses). On D6 of test phase, for reversal 3, the positions were reversed again, however, the 20%-bar was modified to give 33% reinforcement (1 out of every 3 presses), and the 75%-bar was modified to give 67% reinforcement (2 out of every 3 presses). Each day started with the prior day's reinforcement schedule for 2 min before switching to the new schedule for 8 min. For each minute, a bar preference score was calculated as the number of presses for the 75% bar during D3 divided by the total number of presses for both bars. The learning rate was calculated as the absolute value of the slope of the line of the bar preference score from minute 2 to minute 6 of the session.

Determination of blood toluene concentration

Rats were placed in toluene inhalation chambers measuring 21 × 27 × 21 cm (LJARI, USA) and exposed to 3000 ppm toluene vapor (toluene group). The toluene vapor concentration inside the chamber was calibrated with a portable toluene gas detector (Cosmos XP-3360II, DOD Technologies, USA). Toluene (99.5%, analytical grade, RCI Labscan, Thailand) was delivered to the chamber via custom-made bubblers attached to air flow regulators (LJARI, USA). The toluene group received six 30-min toluene exposure sessions on alternating days, similar to the conditioning schedule used for the behavioral studies (Fig. 1B). Control rats were only exposed to air. Blood samples (500 µl) were collected from the tail vein into heparin tubes 10 min after the first toluene exposure (D1), 10 min after the last toluene exposure (D6), and 24 h after the last toluene exposure (D7 or abstinence D1). From each tube, triplicates of 100-µL heparinized blood samples were immediately transferred to a headspace vial that was capped and sealed with parafilm before storage at 4 °C. Gas chromatographic analysis of the samples using a validated protocol was conducted the next day. The concentration of toluene in the blood samples was determined using static headspace gas chromatography with flame ionization detector (HS-GC-FID) using the GC-FID Nexus 2030 with HS-20 headspace autosampler and LabSolutions Post-Run Software (Shimadzu Corp., Japan). The GC column used was a silicone capillary column (SH-I-624Sil MS, 30 m × 0.25 mm × 1.40 µm, Shimadzu Asia Corp., Japan). Nitrogen carrier gas flow rate was set at 200 mL/min, headspace oven temperature was set at 80 °C, and column oven temperature program commenced at 50 °C for 2 min and ramped up to 175 °C at a rate of 25 °C/min, holding the final temperature for 5 min. FID was set at 250 °C.

A five-point calibration curve was used to estimate the levels of toluene in the blood. The calibration solutions in the concentration range of 0 to 25 µg/mL toluene were prepared using blood from naïve animals, spiked with appropriate amounts of stock standard toluene solutions (Chem-Supply Pty Ltd, Australia). Samples and calibration solutions in each headspace vial contained 5 µg/mL isobutanol (J.T Baker, USA) as internal standard (IS) and were dissolved in 2% ethanol. Deionized water was added to samples and calibration solutions to achieve a final volume of 500 µL. Calibration curves were constructed by plotting the peak area ratio of toluene to IS of each calibration solution against its toluene concentration.

Statistical analysis

Data were analyzed using Prism version 10.1.1 (GraphPad Software, USA). Two-way repeated measures ANOVA (for complete datasets) or mixed-effects analysis (for incomplete datasets) was used to analyze data for all behavioral tests and blood toluene levels, followed by post-hoc Dunnett's test (for experiments with 3 groups: both toluene groups compared to air control group) or Šidák's test (for experiments comparing 2 datasets: males compared to females; habituation compared to test scores in NOR, sociability, and social novelty; 3000 ppm toluene group compared to air control group in reversal learning). Differences were considered significant for $p < 0.05$. Data are presented as a mean and standard error of the mean (SEM). Linear regression analysis was performed on the GC calibration data using Microsoft Excel.

Results

Rats were exposed to air in the white chamber and toluene vapor in the black chamber for 30 min per day, for 6 days distributed over a 12-day period (Fig. 1A). We administered toluene vapor at exposure concentrations of 1500 ppm or

3000 ppm through a vapor delivery system that maintained toluene levels constant across a given session (Supplementary Fig. 1). All behavioral tests were performed during the test (drug-free) phase (Fig. 1B).

Prior toluene exposure increased locomotion in males, but not in females

We measured the effects of repeated toluene exposure on locomotion on days 3, 7, and 21 of test phase (D3, D7, and D21) in an open field arena for 10 min (Fig. 2A). In control rats (Fig. 2B), we observed a pronounced sex difference in locomotion, with males showing significantly less distance travelled compared to females at all three timepoints (D3 males, females: 52.01 m, 70.07 m; D7 males, females: 46.38 m, 69.95 m; D21 males, females: 49.90 m, 74.71 m). Two-way repeated measures ANOVA showed a main effect of sex ($F_{1,28} = 32.36$ $p < 0.001$) and significant between-group differences (post-hoc tests males vs. females on D3, D7, and D21 of test phase: all p's < 0.001).

Due to this sex difference in controls, we next analyzed the effects of toluene on locomotion in males and females separately. Males given 3000 ppm toluene exhibited significantly increased locomotion on D7 (58.08 m) and D21 (62.15 m) of test phase compared to controls (46.38 m, 49.90 m) (Fig. 2C, two-way RM ANOVA main effect of drug: $F_{2,40} = 3.761$ $p = 0.032$; post-hoc tests control vs. 3000 ppm toluene on D7: $p = 0.023$; D21: $p = 0.017$). There was no significant effect of 1500 ppm of toluene in males (post-hoc test control vs. 1500 ppm toluene: $p = 0.186$). Female rats showed no significant effect of prior toluene exposure at either exposure concentration (Fig. 2D, two-way RM ANOVA main effect of drug: $F_{2,42} = 2.324$ $p = 0.110$).

Prior toluene exposure resulted in long-lasting CPP in females, but not in males

We first performed an analysis of males and females combined, as both male and female rats underwent the CPP protocol at the same time (Fig. 3A, B). During preconditioning, groups were matched for CPP ratio scores (control = 0.52, 1500 ppm = 0.52, 3000 ppm = 0.53). Two-way RM ANOVA of D1, D8, and D22 of CPP test showed a main effect of drug ($F_{2,87} = 5.600$ $p = 0.005$) but not of test day ($p = 0.500$) or drug × test day interaction ($p = 0.954$). Post-hoc analysis to determine main group differences across all test days revealed significantly increased CPP scores in rats exposed to both 1500 and 3000 ppm toluene compared to controls (control vs 1500 ppm toluene $p = 0.027$; control vs 3000 ppm toluene $p = 0.005$). We then tested whether these group differences were significant at each test day and found

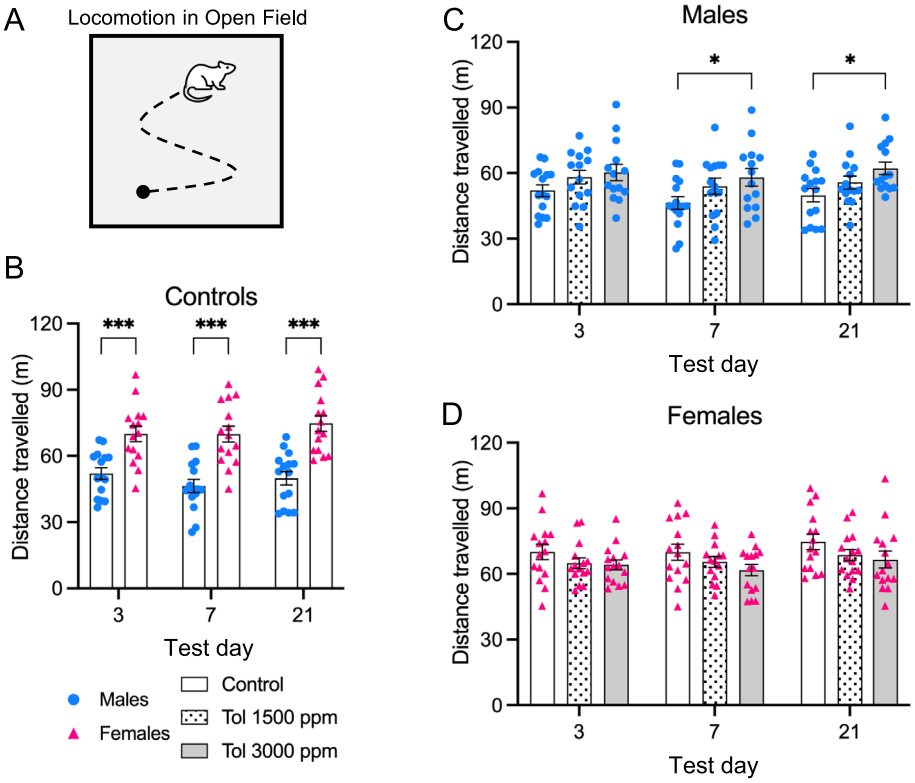

Fig. 2 Effects of prior toluene exposure on locomotion. **A** Locomotion was assessed in an open field over 10 min. **B** Male control rats showed significantly less locomotion compared to female control rats on D3, D7, and D21 of test phase ($n = 15$ per group). **C** Male rats exposed to 3000 ppm toluene ($n = 14$) showed significantly increased locomotion compared to controls ($n = 15$) on D7 and D21 of test phase. Males exposed to 1500 ppm toluene ($n = 14$) did not differ from controls. **D** Toluene had no effect on locomotion in female rats ($n = 15$ per group). * $p < 0.05$; *** $p < 0.001$

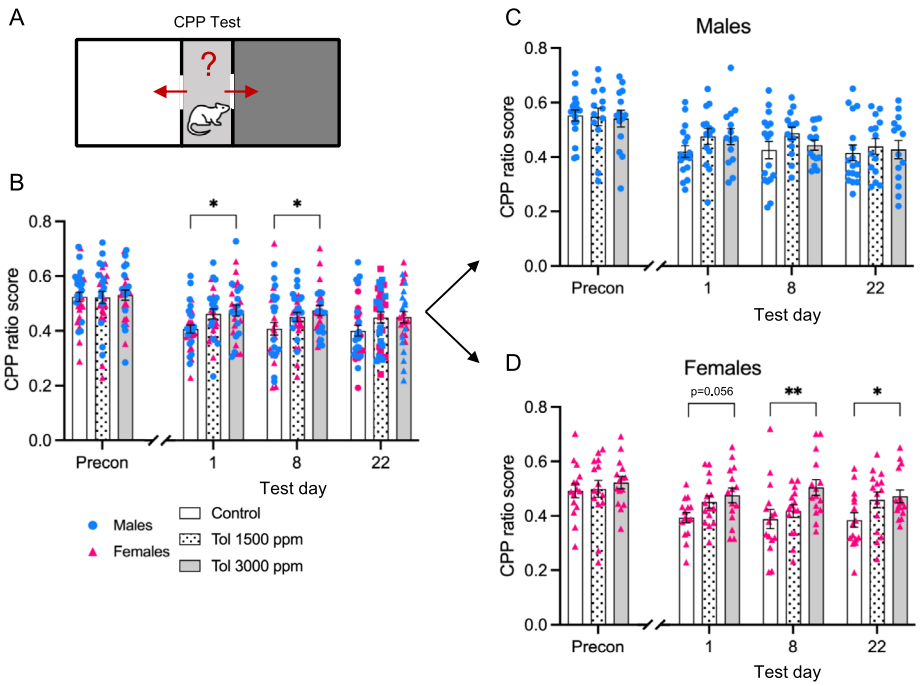

Fig. 3 Effects of prior toluene exposure on conditioned place preference (CPP) ratio score**. A** Representation of the CPP test. **B** Analysis of combined sexes showed significantly increased CPP ratio scores for rats exposed to 3000 ppm toluene ($n=29$) compared to controls ($n=32$) on D1 and D8 of test phase, but not in rats exposed to 1500 ppm toluene ($n=29$) (two-way RM ANOVA main effect of drug $p=0.005$). **C** Analysis of male rats did not reveal a significant increase in CPP ratio score for either toluene exposure concentration (two-way RM ANOVA main effect of drug $p=0.213$; control $n=17$, toluene 1500 ppm $n=14$, toluene 3000 ppm $n=14$). **D** Analysis of female rats showed significantly increased CPP ratio scores for rats exposed to 3000 ppm toluene compared to controls on D8 and D22 of test phase and a trend on D1 (two-way RM ANOVA main effect of drug $p=0.007$). Females exposed to 1500 ppm toluene did not significantly differ from controls ($n=15$ per group). * $p<0.05$; ** $p<0.01$

that the 3000 ppm group showed significantly increased CPP ratio scores on D1 (0.48) and D8 (0.48) of test phase compared to controls (0.41, 0.41) (control vs. 3000 ppm toluene on D1: p = 0.024; D8: p = 0.026; D22: p = 0.120). No significant increase in CPP ratio scores was observed for 1500 ppm at any timepoint (D1: 0.46, D8: 0.45, D21: 0.45; post-hoc tests D1: $p=0.073$; D8: $p=0.192$; D22: $p=0.135$).

When male rats were analyzed separately (Fig. 3C), the CPP increase failed to reach significance at either exposure concentration of toluene compared to controls (two-way RM ANOVA main effect of drug: $F_{2,42}=1.603$ $p=0.213$; test day: $p=0.289$; drug x test day interaction: $p=0.806$). However, in female rats (Fig. 3D), two-way RM ANOVA showed a main effect of drug ($F_{2,42}=5.638$ $p=0.007$) but not of test day ($p=0.984$) or drug × test day interaction ($p=0.467$). Post-hoc analysis to determine main group differences across all test days revealed significantly increased CPP scores in rats exposed to 3000 ppm toluene compared controls ($p=0.003$), but not in rats exposed to 1500 ppm ($p=0.117$). We then tested whether these group differences are significant at each timepoint and found that the 3000 ppm group showed significantly increased CPP ratio scores on D8 (0.50) and D22 (0.47) of test phase compared to controls (0.39, 0.39) (control vs. 3000 ppm toluene on D8: $p=0.005$; D22: $p=0.041$). There was a trend towards significance for D1 ($p=0.056$). We also analyzed our data using a CPP difference score (time in black chamber minus time in white chamber) (McKendrick and Graziane 2020). Our statistical findings using this score were similar to those using the ratio score (see Supplementary Fig. 2). There was no effect of toluene on the number of entries in each chamber for either males or females (data not shown). Thus, after separating the sexes, we observed significant CPP in females only.

Prior toluene exposure did not alter anxiety-like behavior, object memory, or preference for social cues

We used two tests to measure anxiety-like behavior during test phase: elevated plus maze (EPM) (Fig. 4A) and open field test (OFT). In the EPM, we found a pronounced sex difference in controls, with females showing significantly less anxiety-like behavior (increased time in the open arms) on D2 and D6 of test phase compared to males (Fig. 4B). This

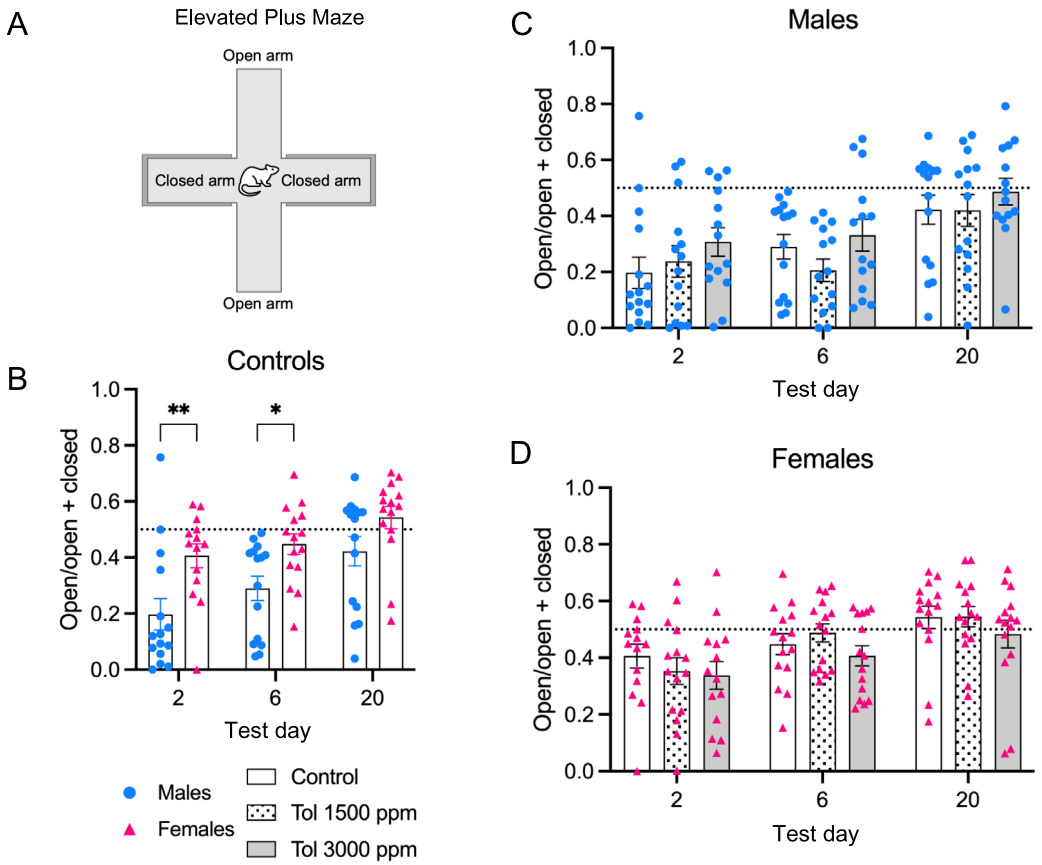

Fig. 4 Lack of toluene effects on anxiety-like behavior. **A** Representation of the elevated plus maze (EPM). **B** Female control rats spent significantly more time in the open arms on D2 and D6 of test phase compared to male control rats ($n = 15$ per group). **C–D** Toluene did not alter the time spent in the open arms in males (control $n = 15$, toluene 1500 ppm $n = 14$, toluene 3000 ppm $n = 14$) or females ($n = 15$ per group). * $p < 0.05$; ** $p < 0.01$

was apparent in the ratio of time spent in the open arms (D2 males, females: 0.20, 0.41; D6 males, females: 0.29, 0.45; D20 males, females: 0.42, 0.54; mixed effects analysis main effect of sex: $F_{1,28} = 10.51$ $p = 0.003$; post-hoc tests males vs. females on D2: $p = 0.007$; D6: $p = 0.046$; D20: $p = 0.180$). However, we did not observe any effect of prior toluene exposure in the EPM in either males (Fig. 4C, two-way RM ANOVA main effect of drug: $F_{2,40} = 1.295$ $p = 0.285$) or females (Fig. 4D, mixed effects analysis main effect of drug: $F_{2,42} = 1.418$ $p = 0.254$). In the OFT, we observed a trend toward less anxiety-like behavior (more time in the center) in control females compared to control males (consistent with the EPM findings) (Supplementary Fig. 3 A, two-way RM ANOVA main effect of sex: $p = 0.068$). As in the EPM, there was no significant effect of toluene exposure on OFT in either males or females (Supplementary Fig. 3 B–C).

We measured novel object recognition memory (NOR) on D7 of test phase (Fig. 5A). An NOR score reflecting the ratio of time spent exploring the novel vs. the familiar object was calculated. We analyzed the first 2 min in the NOR test because the memory effect in controls (increase in the NOR score from habituation to test) reached significance during the first 2 min ($p < 0.001$), but not during the last 2 min ($p = 0.078$). Analysis of the combined sexes showed an increase in the NOR score from habituation to test in all three groups, indicating intact object memory (Fig. 5B, test day NOR score control: 0.60, 1500 ppm toluene: 0.62, 3000 ppm toluene: 0.62; post-hoc tests habituation vs. test control: $p = 0.005$, 1500 ppm toluene: $p = 0.005$, 3000 ppm toluene: $p < 0.001$). There was no effect of exposure to either concentration of toluene on NOR (two-way RM ANOVA main effect of drug: $F_{2,87} = 0.731$ $p = 0.484$; phase: $F_{1,87} = 45.48$ $p < 0.001$; interaction: $F_{2,87} = 1.335$ $p = 0.268$). When the sexes were analyzed separately, exposure to toluene did not affect object recognition memory in either males (Supplementary Fig. 4 A, two-way RM ANOVA main effect of drug: $F_{2,42} = 0.726$ $p = 0.490$; interaction: $F_{2,42} = 0.072$ $p = 0.930$) or females (Supplementary Fig. 4 B, two-way RM ANOVA main effect of drug: $F_{2,42} = 0.154$ $p = 0.857$;

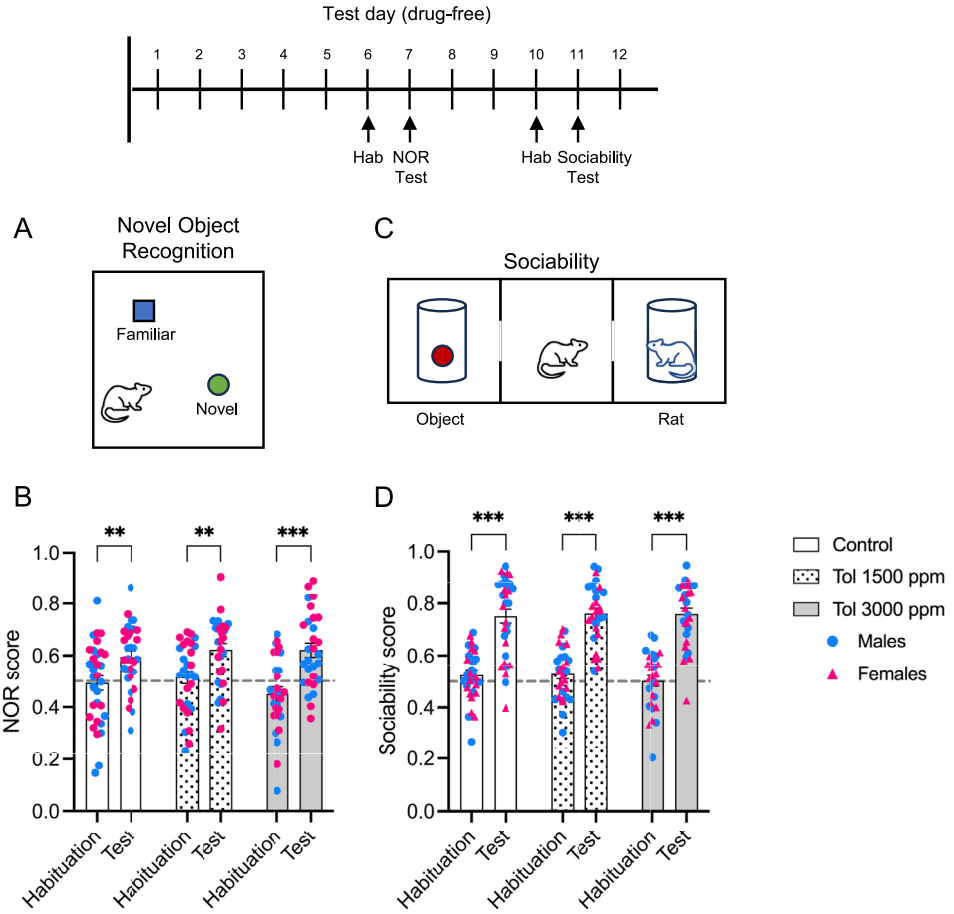

Fig. 5 Lack of toluene effects on object memory and sociability. **A** Representation of the novel object recognition (NOR) test. **B** On D7 of test phase, analysis of the sexes combined showed an increase in the NOR score from habituation to test, indicating intact object memory (control $n=32$, toluene 1500 ppm $n=29$, toluene 3000 ppm $n=29$). There was no effect of toluene on NOR (two-way RM ANOVA main effect of drug $p=0.484$; phase $p<0.001$; interaction $p=0.268$). **C**) Representation of the sociability test. **D**) On D11 of test phase, analysis of the sexes combined showed an increase in sociability score from habituation to test, indicating a preference for social cues (control $n=32$, toluene 1500 ppm $n=29$, toluene 3000 ppm $n=29$). There was no effect of toluene on sociability (two-way RM ANOVA main effect of drug p = 0.825; phase $p<0.001$; interaction $p=0.733$). ** $p<0.01$; *** $p<0.001$

interaction: $F_{2,42}=1.999$ p = 0.148). There was no effect of toluene on exploration time during the NOR test (Supplementary Fig. 5 A).

We next performed a sociability test on D11 of test phase, in which rats explored side chambers containing either a wooden object or a same-sex rat (Fig. 5C). Analysis of the sexes combined showed an increase in sociability score from habituation to test, indicating a preference for social cues (Fig. 5D, test day sociability score control: 0.75, 1500 ppm toluene: 0.76, 3000 ppm toluene: 0.76; post-hoc tests habituation vs. test: all p's < 0.001). There was no effect of either toluene concentration on sociability (two-way RM ANOVA main effect of drug: $F_{2,87}=0.192$ p = 0.825; phase: $F_{1,87}=207.6$ p < 0.001; interaction: $F_{2,87}=0.312$ p = 0.733). When the sexes were analyzed separately, exposure to toluene did not affect sociability in either males (Supplementary Fig. 4 C, two-way RM ANOVA main effect of drug: $F_{2,42}=0.044$ p = 0.957; interaction: $F_{2,42}=0.239$ p = 0.788) or females (Supplementary Fig. 4 D, two-way RM ANOVA main effect of drug: $F_{2,42}=0.218$ p = 0.805; interaction: $F_{2,42}=0.350$ p = 0.707). There was no effect of toluene on exploration time during the sociability test (Supplementary Fig. 5 B).

Prior toluene exposure impaired preference for social novelty

On D12 of test phase, the day following the sociability test, we again presented rats with a choice in the 3-chamber social apparatus. One side contained the same rat from the day before, whereas the other side contained a novel rat of the same sex (Fig. 6A). For this test, we used the last 5 min of the session because the social novelty effect in controls with both sexes combined (increased time spent with the novel rat) was observed only during the last 5 min (p < 0.001), but not during the first 5 min (p = 0.786). There was a significant interaction between drug and phase (two-way RM ANOVA main effect drug: $F_{2,33}=1.688$ p = 0.201; phase: $F_{1,33}=0.924$ p = 0.344; interaction: $F_{2,33}=9.408$ p < 0.001). Controls showed a significant increase in the social novelty score (Fig. 6B, habituation: 0.47, test: 0.68, post-hoc test: p < 0.001),

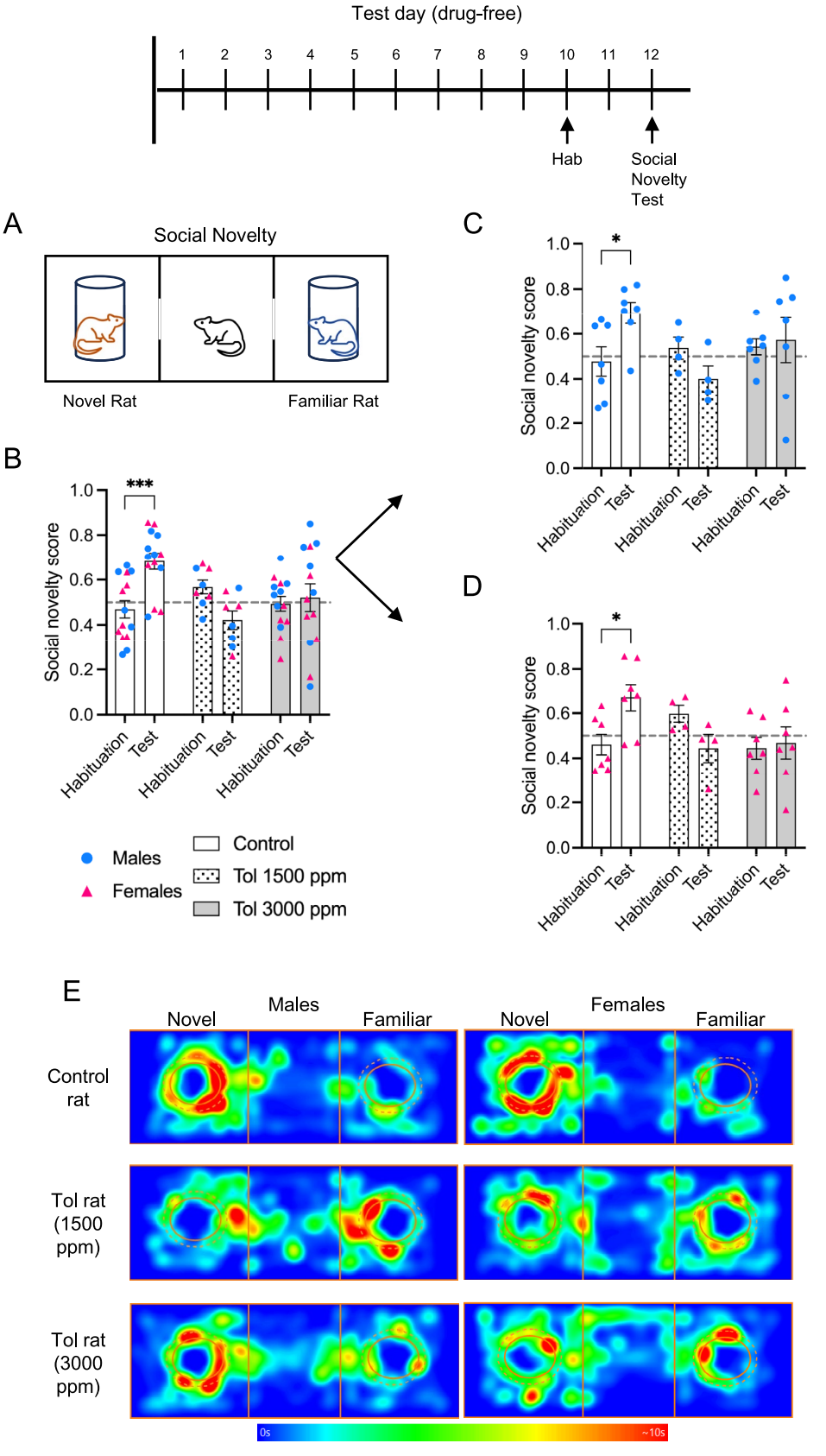

Fig. 6 Effects of prior toluene exposure on preference for social novelty. **A** Representation of the social novelty test performed on D12 of test phase. **B** Analysis of the sexes combined showed a significant increase in the social novelty score from habituation to test in controls ($n=14$), indicating preference for social novelty. Rats exposed to 1500 ppm toluene ($n=8$) or 3000 ppm toluene ($n=14$) did not show an increase in the social novelty score (two-way RM ANOVA main effect of drug $p=0.201$; phase $p=0.344$; interaction $p<0.001$). **C**) Analysis of male rats showed an increase in the social novelty score in controls but not in the toluene groups (control $n=7$, toluene 1500 ppm $n=4$, toluene 3000 ppm $n=7$; two-way RM ANOVA main effect of drug $p=0.360$; phase $p=0.475$; interaction $p=0.043$). **D** Analysis of female rats showed an increase in the social novelty score in controls but not in the toluene groups (control $n=7$, toluene 1500 ppm $n=4$, toluene 3000 ppm $n=7$; two-way RM ANOVA main effect of drug $p=0.190$; phase $p=0.584$; interaction $p=0.026$). **E** Heat maps of representative rats from each group showing the position of the test animal (head) during the social novelty test. * $p<0.05$; *** $p<0.001$

indicating preference for social novelty. Rats exposed to 1500 ppm toluene (habituation: 0.57, test: 0.42; post-hoc test: $p = 0.110$) or 3000 ppm toluene (habituation: 0.49, test: 0.52; post-hoc test: $p = 0.937$) did not show an increase in the social novelty score. Between-group comparison during test phase showed significantly lower social novelty scores in the 1500 ppm ($p = 0.001$) and 3000 ppm toluene ($p = 0.012$) groups when compared to the control group.

Analyzing the sexes separately yielded similar results. Male rats showed an increase in the social novelty score in controls (Fig. 6C, habituation: 0.48, test: 0.69; two-way RM ANOVA main effect drug: $F_{2,15} = 1.096$ $p = 0.360$; phase: $F_{1,15} = 0.536$ $p = 0.475$; interaction $F_{2,15} = 3.913$ $p = 0.043$; post-hoc test: $p = 0.041$) but not in the toluene groups (1500 ppm toluene habituation: 0.54, test: 0.40, post-hoc test: $p = 0.498$, 3000 ppm toluene habituation: 0.54, test: 0.57, post-hoc test: $p = 0.975$). However, we observed that male rats exposed to 1500 ppm toluene, but not 3000 ppm, showed significantly lower social novelty scores compared to controls (1500 ppm toluene: $p = 0.015$; 3000 ppm toluene: $p = 0.300$). Female controls also showed an increase in the social novelty score (Fig. 6D, habituation: 0.46, test: 0.67, two-way RM ANOVA main effect drug $F_{2,15} = 1.861$ $p = 0.190$; phase $F_{1,15} = 0.313$ $p = 0.584$; interaction: $F_{2,15} = 4.700$ $p = 0.026$; post-hoc test: $p = 0.034$) but not in the toluene groups (1500 ppm toluene habituation: 0.60, test: 0.44, post-hoc test: $p = 0.343$, 3000 ppm toluene habituation: 0.44, test: 0.47, post-hoc test: $p = 0.985$). Furthermore, females in both toluene groups showed significantly lower scores than controls (1500 ppm toluene: $p = 0.032$; 3000 ppm toluene: $p = 0.025$).

This effect of toluene on social preference is apparent in the heat maps (Fig. 6E) of representative rats from each experimental group. Importantly, toluene did not alter the total time exploring both chambers (Supplementary Fig. 5 C). This impairment in social preference was unlikely due to toluene-induced anosmia because we observed that a separate group of rats exposed to 3000 ppm toluene for 6 days were unimpaired in their preference for peanut oil odor (vs. no odor) during day 13 of test phase (control $n = 6$, 3000 ppm toluene $n = 6$; unpaired t-test comparing odor preference between control and 3000 ppm toluene groups: $t_{10} = 0.063$ $p = 0.951$).

Prior toluene exposure slowed reversal learning

In a separate group of rats, we tested reversal learning in an appetitive bar pressing task using food reward (Stephenson-Jones et al. 2016) during D2–D6 of test phase (Fig. 7A). On D2–D3, rats were trained to discriminate between one bar that yielded no food pellets (0%) from a bar that yielded a pellet 75% of the time (0%—75%, 3 out of every 4 presses). On D4–D6, we assessed reversal behavior with increasing difficulty: D4 (75%—0%, reversal 1), D5 (20%—75%, reversal 2), D6 (67%—35%, reversal 3). Each day started with the prior day's reinforcement schedule for 2 min before switching to the new schedule for 8 min. For total bar pressing rates (both bars combined), we found a significant interaction of drug and phase (Supplementary Fig. 6 A, two-way RM ANOVA main effect of drug: $F_{1,35} = 0.115$ $p = 0.737$; phase: $F_{4,140} = 53.74$ $p < 0.001$; interaction: $F_{4,140} = 2.488$ $p = 0.046$; post-hoc tests control vs. toluene: all p's ≥ 0.441), suggesting that the toluene group increased their rate of bar pressing across phases more than the control group.

In reversal learning (Fig. 7B), toluene did not impair the ability of rats to perform reversal 1, as indicated by an equivalent decrease in bar preference score in both control and toluene rats. However, during reversal 2, toluene rats learned more slowly than controls, and in reversal 3, toluene rats reversed faster than controls. We observed a significant main effect of drug exposure across the two more difficult reversals (from the start of reversal 2 until the end of reversal 3) (two-way RM ANOVA main effect of drug: $F_{1,35} = 4.237$ $p = 0.047$; minute: $F_{17,595} = 40.67$ $p < 0.001$; interaction: $F_{17,595} = 0.514$ $p = 0.947$). For the learning rate, we found a significant interaction between groups and reversal phase (Supplementary Fig. 6 B), indicating that toluene rats were slower than controls in reversal 2 and faster in reversal 3 (two-way RM ANOVA main effect of drug: $F_{1,35} = 0.009$ $p = 0.923$; phase: $F_{1,35} = 10.52$ $p = 0.003$; interaction: $F_{1,35} = 4.441$ $p = 0.042$). Analysis of males and females separately showed no significant effect of toluene on the rate of reversal learning (Supplementary Fig. 6 C–F).

Measurement of blood levels of inhaled toluene

In a separate group of adolescent rats (9 males and 9 females), we used gas chromatography to measure the blood levels of toluene in rats exposed to 3000 ppm of toluene vapor (Supplementary Fig. 7 A–B). Rats were exposed to toluene (or air) across 6 alternating days and blood samples were analyzed at D1 exposure, D6 exposure, and D7 (drug-free day). Blood levels of toluene were equivalent on D1 and D6 (mixed effects analysis main effect of time: $F_{1,9} = 2.953$ $p = 0.120$). On D7, there was no detectable toluene in both groups. There were no significant sex differences at any timepoint (D1 male, female: 8.210 µg/mL, 9.586 µg/mL; mixed effects analysis main effect of sex: $F_{1,10} = 0.209$ $p = 0.657$) and (D6 male, female: 7.338 µg/mL, 7.095 µg/

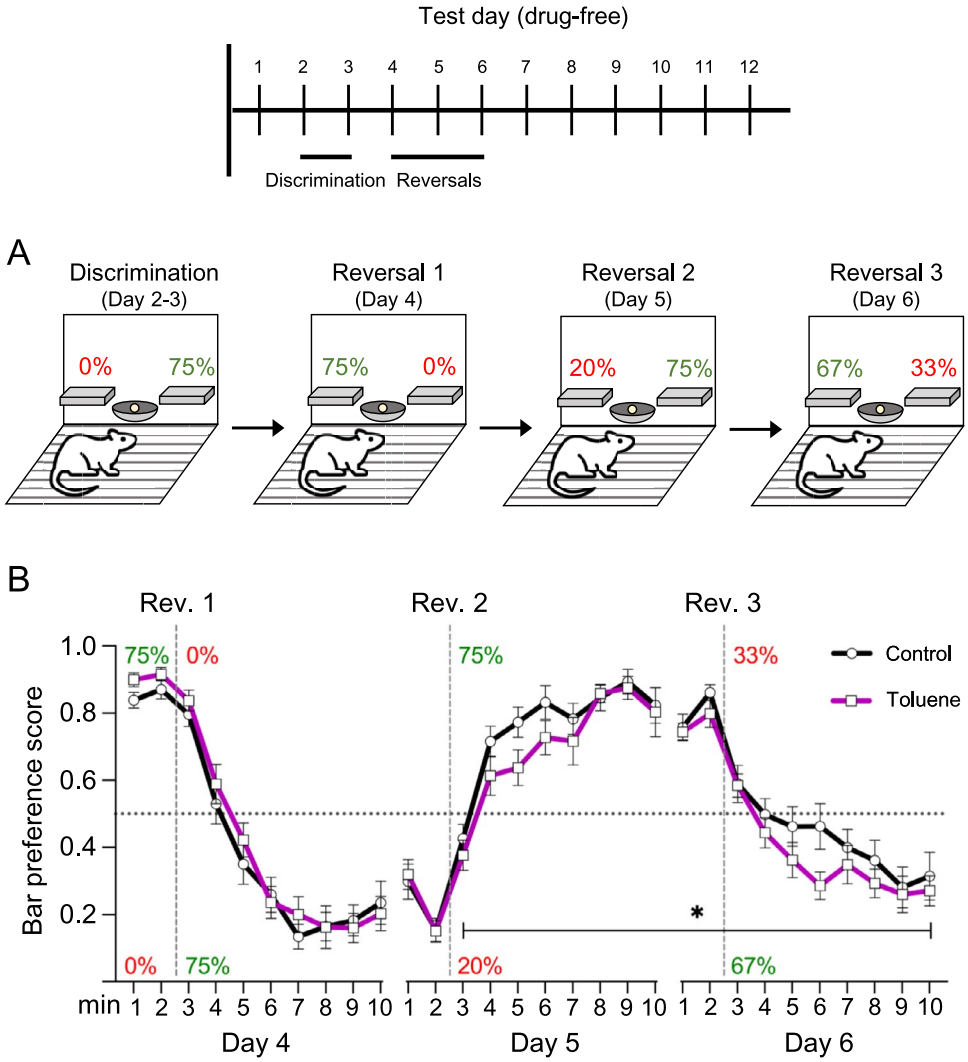

Fig. 7 Effects of prior toluene exposure on reversal learning. **A** Representation of the reversal learning task performed during D2–D6 of test phase. **B** Rats exposed to toluene were slower to reverse on reversal 2, and faster to reverse on reversal 3 compared to controls (control $n=21$, toluene 3000 ppm $n=16$) (two-way RM ANOVA main effect of drug $p=0.047$; minute $p<0.001$; interaction $p=0.947$). * $p<0.05$

mL). Thus, the sex differences in behavior we observed were not due to a difference in toluene levels in the blood. We did not detect toluene in the blood of air controls at any timepoint.

Discussion

We report long-lasting effects of toluene on specific behaviors during the abstinence period in adolescent male and female rats. We found that 3000 ppm toluene vapor induced CPP lasting at least 22 days during abstinence in females, but not in males. In contrast, toluene increased locomotion in males but not in females. Toluene had no effect on anxiety-like behavior or object memory, but impaired preference for social novelty and reversal learning. Thus, we present a comprehensive behavioral evaluation of the long-term effects of toluene, incorporating sex differences and addressing disagreements in the literature.

CPP is a well-established procedure that measures conditioned rewarding effects of drugs and has been posited to reflect drug seeking behavior (McKendrick et al. 2020; O'Neal et al. 2022). Toluene has been previously reported to induce CPP in male rodents, however, no prior study examined CPP in female rodents, despite clinical data showing inhalant use in females (Crossin and Arunogiri 2020). We found a significant effect of toluene on CPP in females that lasted 22 days into abstinence, which is longer than prior reports in males of 7 days duration (Wayman and Woodward 2018).

We were surprised to observe no significant toluene CPP in males after 6 conditioning sessions. Previous studies using 6 conditioning sessions have demonstrated toluene CPP in male rats at exposure concentrations of 2000–5000 ppm (Gerasimov et al. 2003; Lee et al. 2006; Wayman & Woodward 2018; but see Lee et al. 2004). Unlike prior studies, we delivered toluene always in the same (black) chamber, which prevented us from

controlling for any conditioned chamber preferences, independent of the drug (Cunningham et al. 2006; McKendrick et al. 2020). Any conditioned aversion to the black chamber would tend to mask the rewarding effects of toluene. It is also possible that a concentration higher than 3000 ppm (e.g., 5000 ppm) would have yielded a significant CPP effect in males. 3000 ppm is considered to be in the low range of typical human inhalant use (Crossin et al. 2019; Hubková et al. 2022; Woodward and Braunscheidel 2023). However, the blood toluene levels that we obtained after exposure to 3000 ppm were in the range observed in human adolescent inhalant users (Thiesen et al. 2007). It is also possible that increasing the duration of exposure to 12 days (Lee et al. 2004; Schiffer et al. 2006) would have yielded a significant effect in males. In contrast to females, males showed increased locomotion during abstinence, a finding consistent with other drugs such as cocaine (Mañas-Padilla et al. 2021). Thus, there may be a sex difference in how toluene history is expressed behaviorally during the abstinence period.

We did not observe increased anxiety-like behavior during prolonged abstinence, similar to prior studies (Lin et al. 2010; Bowen et al. 2018). With respect to novel object memory, we found no impairments, which disagrees with two prior reports (Lin et al. 2010; Montes et al. 2017). However, compared to our study, these studies used a longer period of exposure (4 weeks vs. 6 days) (Montes et al. 2017) or a higher exposure concentration (63 µg/mL vs. 8 µg/mL in blood) (Lin et al. 2010), suggesting that a higher exposure may yield a memory impairment.

With respect to social behavior, we found that prior toluene exposure did not impair sociability but eliminated preference for social novelty, in both males and females. This suggests that toluene-exposed rats preferred a social stimulus vs. a non-social stimulus, but did not prefer a novel rat vs. a familiar rat. This deficit was unlikely due to a loss of memory, because their novel object memory was intact. Rather, toluene appeared to decrease interest in relevant social cues. Our finding agrees with a prior toluene study that found decreased interaction with a novel mouse (Lin et al. 2010). This effect may be unique to toluene, as deficits in social novelty preference were not observed with other drugs of abuse, such as cocaine, ethanol, fentanyl, or morphine (Morisot et al. 2018; Sidhu et al. 2018; Fujii et al. 2018; Piccin and Contarino 2020).

We found modest cognitive impairments in our reversal learning task. Toluene rats showed intact reversal learning during an easy reversal task (75% vs. 0%) but were slower in learning the correct lever in a more difficult task (20% vs. 75%), suggesting reduced discrimination of reward probabilities. The impairment in reversal 2 (less time at the correct bar) may have carried over to the following day (more likely to leave that bar, i.e., the correct bar in reversal 2), hence leading to a faster reversal 3. Prior studies of toluene in adults also showed slower reversal learning (Dick et al. 2014; Furlong et al. 2016) as well as impairments in similar tasks varying reward probability such as contingency degradation (Furlong et al. 2016) and delayed discounting (Braunscheidel et al. 2019).

Our findings resemble some of the deficits seen in human adolescents with IUD. Male teenagers showed high relapse rates after attempts to abstain from inhalant use (Verma et al. 2011; Dhawan et al. 2015). However, data on females are lacking despite a higher prevalence of inhalant use in females in some studies (Crossin and Arunogiri 2020). Thus, there is a pressing need to include females in future studies of IUD. Regarding other behaviors, deficits in cognitive flexibility (similar to our reversal learning deficits) persist for long periods of time (Dingwall et al. 2011; Yuncu et al. 2015). The impairment in social preference we found has not been described clinically, however, inhalant users have a higher prevalence of antisocial behavior (Howard et al. 2010). Our social task was designed to model social deficits observed in autism spectrum disorder (ASD) in mice (Moy et al. 2004), and our results suggest the interesting possibility that the abstinence period following inhalant use may be associated with some features of ASD.

Future clinical studies on toluene-induced cognitive and social deficits are warranted, as these effects could interfere with treatment. Rehabilitation programs could consider incorporating cognitive training (Verdejo-Garcia et al. 2023) and social-based interventions (Meyers et al. 2011; Venniro et al. 2021), with the aim of reducing persistent drug seeking and relapse.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00213-024-06731-5.

Acknowledgements We thank Szarina Krisha K. Ko, Herbert Montalban, Jay-ann Tomagan, and Kristine Anne A. Ladines for their assistance with running the experiments, and Arturo Bermejo III for administrative assistance. We thank Jose Rodriguez-Romaguera, Christian Bravo-Rivera, and John Woodward for guidance and donations of equipment. We also like to thank Freddyson Martinez-Rivera and Yavin Shaham for comments on the data and on an earlier version of the manuscript. This research was supported by the Department of Science and Technology—Philippine Council for Health Research and Development (DOST-PCHRD) Grants-In-Aid grant number DOST2020-04-A1-265. This is the inaugural publication from the Laboratory of Addiction Neuroscience Research at the University of the Philippines Manila - National Institutes of Health.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit

to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

References

Bowen SE, Hannigan JH, Davidson CJ, Callan SP (2018) Abstinence following toluene exposure increases anxiety-like behavior in mice. Neurotoxicol Teratol 65:42–50. https://doi.org/10.1016/j.ntt.2017.12.010

Braunscheidel KM, Okas MP, Hoffman M et al (2019) The Abused Inhalant Toluene Impairs Medial Prefrontal Cortex Activity and Risk/Reward Decision-Making during a Probabilistic Discounting Task. J Neurosci 39:9207. https://doi.org/10.1523/JNEUROSCI.1674-19.2019

Crossin R, Arunogiri S (2020) Harms associated with inhalant misuse in adolescent females - a review of the pre-clinical and clinical evidence. Drug Alcohol Depend 216:108232. https://doi.org/10.1016/j.drugalcdep.2020.108232

Crossin R, Lawrence AJ, Andrews ZB et al (2019) Growth changes after inhalant abuse and toluene exposure: A systematic review and meta-analysis of human and animal studies. Hum Exp Toxicol 38:157–172. https://doi.org/10.1177/0960327118792064

Cruz SL, Bowen SE (2021) The last two decades on preclinical and clinical research on inhalant effects. Neurotoxicol Teratol 87:106999. https://doi.org/10.1016/j.ntt.2021.106999

Cruz SL, Rivera-García MT, Woodward JJ (2014) Review of Toluene Actions: Clinical Evidence, Animal Studies, and Molecular Targets. J Drug Alcohol Res 3:1–8. https://doi.org/10.4303/jdar/235840

Cunningham CL, Gremel CM, Groblewski PA (2006) Drug-induced conditioned place preference and aversion in mice. Nat Protoc 1:1662–1670. https://doi.org/10.1038/nprot.2006.279

Dhawan A, Chopra A, Ambekar A, Ray R (2015) Treatment Seeking Behavior of Inhalant Using Street Children: Are We Prepared to Meet Their Treatment Needs. Indian J Psychol Med 37:282–287. https://doi.org/10.4103/0253-7176.162918

Dick ALW, Axelsson M, Lawrence AJ, Duncan JR (2014) Specific impairments in instrumental learning following chronic intermittent toluene inhalation in adolescent rats. Psychopharmacology 231:1531–1542. https://doi.org/10.1007/s00213-013-3363-7

Dingwall KM, Maruff P, Fredrickson A, Cairney S (2011) Cognitive recovery during and after treatment for volatile solvent abuse. Drug Alcohol Depend 118:180–185. https://doi.org/10.1016/j.drugalcdep.2011.03.017

ESPAD Group (2020) ESPAD Report 2019: Results from the European School Survey Project on Alcohol and Other Drugs. European Monitoring Centre for Drugs and Drug Addiction

Fujii K, Koshidaka Y, Adachi M, Takao K (2018) Effects of chronic fentanyl administration on behavioral characteristics of mice. Neuropsychopharmacol Rep 39:17–35. https://doi.org/10.1002/npr2.12040

Funada M, Sato M, Makino Y, Wada K (2002) Evaluation of rewarding effect of toluene by the conditioned place preference procedure in mice. Brain Res Protoc 10:47–54. https://doi.org/10.1016/S1385-299X(02)00182-4

Furlong TM, Duncan JR, Corbit LH et al (2016) Toluene inhalation in adolescent rats reduces flexible behaviour in adulthood and alters glutamatergic and GABAergic signalling. J Neurochem 139:806–822. https://doi.org/10.1111/jnc.13858

Gerasimov MR, Collier L, Ferrieri A et al (2003) Toluene inhalation produces a conditioned place preference in rats. Eur J Pharmacol 477:45–52. https://doi.org/10.1016/j.ejphar.2003.08.022

Howard MO, Perron BE, Vaughn MG, et al (2010) Inhalant use, inhalant-use disorders, and antisocial behavior: findings from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC). J Stud Alcohol Drugs 71:201–209. https://doi.org/10.15288/JSAD.2010.71.201

Hubková B, Birková A, Čižmárová B (2022) Toluene Abuse. In: Patel VB, Preedy VR (eds) Handbook of Substance Misuse and Addictions: From Biology to Public Health. Springer International Publishing, Cham, pp 2499–2522

Jia W, Kawahata I, Cheng A et al (2023) Amelioration of Nicotine-Induced Conditioned Place Preference Behaviors in Mice by an FABP3 Inhibitor. Int J Mol Sci 24:6644. https://doi.org/10.3390/ijms24076644

Lee DE, Schiffer WK, Dewey SL (2004) Gamma-vinyl GABA (vigabatrin) blocks the expression of toluene-induced conditioned place preference (CPP). Synapse 54:183–185. https://doi.org/10.1002/syn.20072

Lee DE, Gerasimov MR, Schiffer WK, Gifford AN (2006) Concentration-dependent conditioned place preference to inhaled toluene vapors in rats. Drug Alcohol Depend 85:87–90. https://doi.org/10.1016/j.drugalcdep.2006.03.013

Lin B-F, Ou M-C, Chung S-S et al (2010) Adolescent toluene exposure produces enduring social and cognitive deficits in mice: An animal model of solvent-induced psychosis. World J Biol Psychiatry 11:792–802. https://doi.org/10.3109/15622970903406234

Lukas M, Bredewold R, Landgraf R et al (2011) Early life stress impairs social recognition due to a blunted response of vasopressin release within the septum of adult male rats. Psychoneuroendocrinology 36:843–853. https://doi.org/10.1016/j.psyneuen.2010.11.007

Mañas-Padilla MC, Ávila-Gámiz F, Gil-Rodríguez S et al (2021) Persistent changes in exploration and hyperactivity coexist with cognitive impairment in mice withdrawn from chronic cocaine. Physiol Behav 240:113542. https://doi.org/10.1016/j.physbeh.2021.113542

McKendrick G, Graziane NM (2020) Drug-Induced Conditioned Place Preference and Its Practical Use in Substance Use Disorder Research. Front Behav Neurosci 14:582147. https://doi.org/10.3389/fnbeh.2020.582147

McKendrick G, Garrett H, Tanniru S et al (2020) A novel method to study reward-context associations and drug-seeking behaviors. J Neurosci Methods 343:108857. https://doi.org/10.1016/j.jneumeth.2020.108857

Meyers RJ, Roozen HG, Smith JE (2011) The Community Reinforcement Approach. Alcohol Res Health 33:380–388

Montes S, Solís-Guillén R del C, García-Jácome D, Páez-Martínez N (2017) Environmental enrichment reverses memory impairment induced by toluene in mice. Neurotoxicol Teratol 61:7–16.https://doi.org/10.1016/j.ntt.2017.04.003

Morisot N, Monier R, Le Moine C et al (2018) Corticotropin-releasing factor receptor 2-deficiency eliminates social behaviour deficits and vulnerability induced by cocaine. Br J Pharmacol 175:1504–1518. https://doi.org/10.1111/bph.14159

Moy SS, Nadler JJ, Perez A et al (2004) Sociability and preference for social novelty in five inbred strains: an approach to assess

autistic-like behavior in mice. Genes Brain Behav 3:287–302. https://doi.org/10.1111/j.1601-1848.2004.00076.x

Netser S, Meyer A, Magalnik H et al (2020) Distinct dynamics of social motivation drive differential social behavior in laboratory rat and mouse strains. Nat Commun 11:5908. https://doi.org/10.1038/s41467-020-19569-0

Nguyen J, O'Brien C, Schapp S (2016) Adolescent inhalant use prevention, assessment, and treatment: A literature synthesis. Int J Drug Policy 31:15–24. https://doi.org/10.1016/J.DRUGPO.2016.02.001

O'Neal TJ, Bernstein MX, MacDougall DJ, Ferguson SM (2022) A Conditioned Place Preference for Heroin Is Signaled by Increased Dopamine and Direct Pathway Activity and Decreased Indirect Pathway Activity in the Nucleus Accumbens. J Neurosci 42:2011–2024. https://doi.org/10.1523/JNEUROSCI.1451-21.2021

Pelham WE, Tapert SF, Zúñiga ML et al (2023) Pandemic-Related Changes in the Prevalence of Early Adolescent Alcohol and Drug Use, 2020–2021: Data From a Multisite Cohort Study. J Adolesc Health 73:338–346. https://doi.org/10.1016/j.jadohealth.2023.02.040

Piccin A, Contarino A (2020) Long-lasting pseudo-social aggressive behavior in opiate-withdrawn mice. Prog Neuropsychopharmacol Biol Psychiatry 97:109780. https://doi.org/10.1016/j.pnpbp.2019.109780

Potrebić M, Pavković Ž, Puškaš N, Pešić V (2022) The Influence of Social Isolation on Social Orientation, Sociability, Social Novelty Preference, and Hippocampal Parvalbumin-Expressing Interneurons in Peripubertal Rats – Understanding the Importance of Meeting Social Needs in Adolescence. Front Behav Neurosci 16:872628. https://doi.org/10.3389/fnbeh.2022.872628

Schiffer WK, Lee DE, Alexoff DL et al (2006) Metabolic correlates of toluene abuse: decline and recovery of function in adolescent animals. Psychopharmacology 186:159–167. https://doi.org/10.1007/s00213-006-0359-6

Shirenova SD, Khlebnikova NN, Krupina NA (2023) Changes in Sociability and Preference for Social Novelty in Female Rats in Prolonged Social Isolation. Neurosci Behav Physiol 53:103–118. https://doi.org/10.1007/s11055-023-01395-8

Sidhu H, Kreifeldt M, Contet C (2018) Affective disturbances during withdrawal from chronic intermittent ethanol inhalation in C57BL/6J and DBA/2J male mice. Alcohol Clin Exp Res 42:1281–1290. https://doi.org/10.1111/acer.13760

Stephenson-Jones M, Yu K, Ahrens S et al (2016) A basal ganglia circuit for evaluating action outcomes. Nature 539:289–293. https://doi.org/10.1038/nature19845

Sun Y, Pan Z, Ma Y (2017) Increased entrances to side compartments indicate incubation of craving in morphine-induced rat and tree shrew CPP models. Pharmacol Biochem Behav 159:62–68. https://doi.org/10.1016/j.pbb.2017.07.007

Thiesen FV, Noto AR, Barros HMT (2007) Laboratory diagnosis of toluene-based inhalants abuse. Clin Toxicol Phila Pa 45:557–562. https://doi.org/10.1080/15563650701365891

Venniro M, Panlilio LV, Epstein DH, Shaham Y (2021) The protective effect of operant social reward on cocaine self-administration, choice, and relapse is dependent on delay and effort for the social reward. Neuropsychopharmacology 46:2350–2357. https://doi.org/10.1038/s41386-021-01148-6

Verdejo-Garcia A, Rezapour T, Giddens E et al (2023) Cognitive training and remediation interventions for substance use disorders: A Delphi consensus study. Addict Abingdon Engl 118:935–951. https://doi.org/10.1111/add.16109

Verma R, Balhara YPS, Dhawan A (2011) Inhalant abuse: An exploratory study. Ind Psychiatry J 20:103–106. https://doi.org/10.4103/0972-6748.102493

Wayman WN, Woodward JJ (2018) Chemogenetic Excitation of Accumbens-Projecting Infralimbic Cortical Neurons Blocks Toluene-Induced Conditioned Place Preference. J Neurosci 38:1462–1471. https://doi.org/10.1523/JNEUROSCI.2503-17.2018

Woodward JJ, Braunscheidel KM (2023) The Effects of the Inhalant Toluene on Cognitive Function and Behavioral Flexibility: A Review of Recent Findings. Addict Neurosci 5:100059. https://doi.org/10.1016/j.addicn.2022.100059

Yates JR, Beckmann JS, Meyer AC, Bardo MT (2013) Concurrent Choice for Social Interaction and Amphetamine using Conditioned Place Preference in Rats: Effects of Age and Housing Condition. Drug Alcohol Depend 129:240–246. https://doi.org/10.1016/j.drugalcdep.2013.02.024

Yuncu Z, Zorlu N, Saatcioglu H et al (2015) Abnormal white matter integrity and impairment of cognitive abilities in adolescent inhalant abusers. Neurotoxicol Teratol 47:89–95. https://doi.org/10.1016/j.ntt.2014.11.009

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.