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# Facilitation of electrical brain self-stimulation behavior by abused solvents

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**Abstract**

Animal models are needed to study the abuse-related behavioral and pharmacological effects of inhaled solvents. Previous studies have suggested that intracranial self-stimulation techniques may be successfully adapted for testing the effects of solvent exposure. The present study aimed to assess the effects of toluene, cyclohexane, acetone, and petroleum benzine (a widely used mixture of hexanes and heptanes) in rats trained to lever press or nose-poke for electrical stimulation delivered through electrodes implanted into the medial forebrain bundle. It was found that toluene, cyclohexane, and benzine but not acetone, increased rates of responding, particularly at the lower stimulation intensities. In another set of experiments utilizing an auto-titration procedure, all tested solvents significantly reduced self-stimulation thresholds. However, only for toluene and benzine were these effects observed at the exposure levels that did not impair rates of operant performance. There may not be such a clear separation of effects for acetone and cyclohexane. Thus, toluene and benzine appear to selectively affect brain reward systems in a manner similar to that for most other abused drugs. Data from intracranial self-stimulation studies of solvents may be useful in abuse potential assessment of individual compounds and for examining neural and behavioral processes involved in inhalant abuse.


*Keywords:* Solvents; Inhalants; Toluene; Cyclohexane; Acetone; Benzine; Brain stimulation reward; Rats

## 1. Introduction

The phenomenon of solvent (inhalant) sniffing is a growing concern in many countries around the world (Kozel et al., 1995). Yet, relatively little is known about the properties of solvents that lead to their abuse (Balster, 1998). One way to increase knowledge in this area is to systematically compare the effects of abused solvents to those of other drugs of abuse that have been more widely studied. Animal models are particularly important in this regard because laboratory-based human research with industrial chemicals are difficult to perform safely. In addition, adequate methods need to be established to screen and analyze the abuse potential of industrial solvents and other chemicals that are likely to be widely used in the household, offices, and industries.

Behavioral studies in the laboratory animals are showing that various solvents produce a range of effects remarkably similar to those of abused drugs of the central nervous system (CNS) depressant class such as barbiturates, benzodiazepines, and ethanol (Evans and Balster, 1991; Balster, 1998). For example, it was shown that 1,1,1-trichlorethane, toluene, and several other solvents substitute for ethanol, pentobarbital, and/or phencyclidine in the drug discrimination studies in mice (Bowen et al., 1999; Rees et al., 1987a,b). Similarly, in animals trained to discriminate toluene injections, methohexital and oxazepam produced toluene-lever responding in a dose-dependent fashion (Knisely et al., 1990). Other effects shared by some abused solvents and CNS depressants include the production of motor impairment, anticonvulsant effects, and anti-anxiety effects (Evans and Balster, 1991). It has been argued that the abuse potential of specific solvent compounds might be assessed in animals by comparing their profile of behavioral and pharmacological effects across a range of procedures that have proven useful for the abuse potential assessment of typical drugs of abuse.

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One behavioral model that has been widely used for the study of abuse-related properties of drugs is electrical self-stimulation of the brain (Kornetsky and Bain, 1992). Drugs of abuse typically facilitate self-stimulation behavior in laboratory subjects with electrodes implanted into brain reward areas (e.g. Bespalov et al., 1999; Schaefer and Michael, 1992). Previous studies conducted in our laboratory in rats have indicated that self-stimulation methods may be adapted for testing the effects of solvent exposure (Yavich and Zvartau, 1994), where it was demonstrated that one of the most widely used and abused solvents, toluene, is capable of facilitating self-stimulation behavior. Thus, exposure to solvents may sensitize the brain reward systems to electrical stimulation and, therefore, self-stimulation techniques can be used to analyze the abuse potential of solvents just as it is done for most other conventional drugs of abuse.

The aim of the present study was to confirm and extend the earlier findings by testing several additional solvents for direct comparison to the effects of toluene. The other materials selected for study were cyclohexane, acetone, and petroleum benzine (a mixture of hexanes and heptanes). Toluene, acetone, cyclohexane, and benzine are known to be present in abused products (e.g. Flanagan and Ives, 1994), although only toluene has been studied extensively for its effects on animal behavior. In addition, two different experimental procedures were used. One of the procedures (rate–intensity protocol) closely followed the design described by Yavich and Zvartau (1994). This procedure was included to provide comparisons with the data generated using another protocol (auto-titration) that offers a rate-independent assessment of the self-stimulation thresholds. Such procedures are thought to avoid potential confounding impact of motor impairment produced by the test compounds. Amphetamine, pentobarbital, ethanol, and many other drugs significantly reduce the self-titrated thresholds in auto-titration procedures (e.g. Schaefer and Michael, 1987, 1988; Seeger et al., 1981). The studies were also designed to examine the role of concentration and/or time course for solvent effects on behavior.

## 2. Materials and methods

### 2.1. Animals

Adult, male, drug and experimentally naïve Wistar rats (State Breeding Farm "Rappolovo," St. Petersburg, Russia) weighing 250–270 g at the time of surgery were used. Animals were housed in groups of three with food and water available ad libitum. All experiments were conducted during the light period of a 12/12-h day–night cycle (09:00–21:00 h). All testing was performed in accordance with the recommendations and policies of the Helsinki Declaration and the U.S. National Institutes of Health Guidelines for the Use of Animals. Experimental protocols were approved by the Ethics Committee of Pavlov Medical University.

### 2.2. Apparatus

Tests were conducted in two standard operant conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA). Chambers were connected to a microcomputer through a MED interface and were controlled by MED-PC software (MED Associates, East Fairfield, VT). Electrical pulses were produced by a constant-current stimulator (PHB-150B; MED Associates, East Fairfield, VT). The electrical stimuli were delivered to the animal through a two-channel electrical swivel assembly (Plastic One, Roanoke, VA), which extended into the test chamber. The electrical stimulus was a 500-ms train of rectangular bipolar waves with a pulse frequency of 100 Hz and a pulse duration of 0.1 ms. Throughout the experiment the electrical stimuli were displayed on the oscilloscope (C1-55, Russia) which permitted the investigator to determine whether or not the stimulator was functioning properly.

In Experiment 1, chambers were equipped with two retractable levers. Each chamber was housed within a ventilated, lightproof, and sound-attenuating enclosure. Ventilator fans provided a constant level of white noise masking extraneous noise and sounds. In Experiment 2, levers were not used because of fire/explosion safety precautions. Each chamber was equipped with two nose-poke manipulanda. Each chamber was housed within a hermetic-sealed solvent exposure box (6BP1-NZh, Russia; volume=0.15 $m^3$).

### 2.3. Surgery

Under pentobarbital anesthesia (55 mg/kg ip), bipolar stainless-steel electrodes of 0.2 mm thickness (Plastic One), insulated except at the tip, were stereotaxically implanted using a David Kopf Micromanipulator. Electrodes were lowered into the left or right medial forebrain bundle of the lateral hypothalamus (coordinates AP: −2.5 mm from bregma, L: 1.9 mm from the midline, V: 8.8 mm from a flat skull, angle: 0, incisor bar: 0). Four stainless-steel screws were fastened to the rat's scull forming a perimeter around the electrode. Dental phosphate cement and acrylic cement were applied to the skull over and around the jeweler's screws and electrode forming a pedestal, which firmly anchored the electrode in place.

### 2.4. Solvent exposures

Toluene, cyclohexane, acetone, and benzine ("calosha" grade) were obtained from ERA-Henkel (Tosno, St. Petersburg). Benzine, sometimes referred to as petroleum benzine or benzin, is one of the common names for the low boiling point fractions of petroleum and is used mainly as the automobile fuel. It should not be confused with benzene, a simple unsubstituted aromatic hydrocarbon ($C_6H_6$). An analysis of the benzine used in this study was conducted using a gas chromatography–mass spectrometry (HP

5988A GC-MS system with an HP 59970A Chemstation). It was found to contain a mixture of heptane (9.5%), isomeric heptanes (78.5%), hexane (6.6%), isomeric hexanes (4.4%), and other hydrocarbons (1%).

Animals were placed in the operant conditioning chambers within the sealed vapor exposure chambers and predetermined amounts of the liquid solvents were introduced into the exposure chambers. Solvents were applied to the bottom of a laboratory pan (15×30 cm) that had been placed into the exposure chamber next to the nontransparent wall of the operant conditioning chamber. To facilitate the evaporation of the solvents, a ventilation fan was placed near the solvent pan and it was constantly working throughout the experiments as well as during the concentration calibration tests. The solvent amounts were calculated to produce the desired exposure concentrations after complete volatilization under standard temperature and pressure (20±1 °C; 760 mmHg). Vaporization times and vapor concentrations were individually monitored for each solvent concentration by means of the HNU-311 portable gas chromatograph with selective photo-ionization detector. At 14,400 ppm, vapor concentrations were as follows: toluene (m.w.=92.1)—54,140 mg/m$^3$, acetone (m.w.=58.1)—34,160 mg/m$^3$, cyclohexane (m.w.=84.2)—49,540 mg/m$^3$, and heptane (principal benzine fraction, m.w.=100.2)—58,900 mg/m$^3$. For various solvents and exposure concentration levels, the complete vaporization occurred after 10–20 min. These values were used to determine the delays between the introduction of the solvent into the exposure chamber and the start of the experimental test session.

### 2.5. Experiment 1: rate–intensity protocol

After 6 days of postoperative recovery, rats were trained to lever press for electrical brain stimulation (starting at 50-μA level in untrained animals) under a continuous reinforcement schedule during the 30-min daily sessions (Monday to Saturday). After the lever-pressing behavior was established, a titration procedure was employed to determine minimal current intensities for maintenance of self-stimulation behavior. During these sessions, the initial current intensity (20 μA above the threshold current intensity determined during the previous training session) was decreased or increased by 5 μA every 1-min interval depending on the response rate during the last 30 s of the preceding 1-min interval. If the response rate was higher than 10 responses per 30 s, the current intensity was decreased for the next 1-min interval. If the response rate was lower than 10 responses per 30 s, the current intensity was increased for the next 1-min interval. The beginning of each 1-min interval was signaled by the response-noncontingent delivery of two single stimulation trains at the intensity to be available for delivery in the next 1-min period. The response rates during the last 30 s of each 1-min interval were recorded and the minimal intensity of current that maintained operant behavior at the level of no less than 10 responses per 30 s (average from three determinations) was taken as a threshold current intensity for that session for that subject.

Once stable levels of responding were established (less than ±10% variation in the threshold current intensity), the threshold titration procedure was supplemented by the rate–intensity tests which occurred immediately following the threshold titration session as well as 60 and 90 min later (a total of three tests each day). For each of these 5-min rate–intensity tests, the current intensity was initially set at the threshold level as just determined and in every following 1-min trial the current was increased by 20 μA while the number of lever presses was recorded.

As soon as the behavior stabilized, various solvents were tested in an order arranged according to a modified Latin Square design ($n$=6 for each solvent concentration level). Animals were exposed to solvents for 1 h immediately after the first rate–intensity test. Tests were conducted twice a week (Wednesdays and Saturdays) provided that the criteria for stable responding were met on the two most recent self-stimulation sessions.

### 2.6. Experiment 2: auto-titration protocol

Beginning 1 week after surgery, rats were trained to poke into one of the two nose-poke manipulanda (the ‘‘stimulation’’ side) in order to receive brain stimulation under a continuous reinforcement schedule. As soon as the stable nose-poke responding was evident (typically, within two 60-min sessions), side switching training sessions were introduced. During these sessions, animals were required to make 100 nose pokes at what was designated the ‘‘stimulation’’ nose-poke manipulandum and then switch to the other side, designated as the ‘‘reset’’ manipulandum for future sessions. After completing a response requirement at the ‘‘reset’’ side, they had to switch back to the ‘‘stimulation’’ side and so on. For the first training session, the response requirement at the ‘‘reset’’ side was set at 100 pokes and was progressively decreased by half until responses on the ‘‘reset’’ manipulandum were not reinforced with electrical stimulation. Current intensity level remained stable throughout these sessions at the level determined during the continuous reinforcement training sessions. These sessions lasted 30–60 min depending on the performance of the animal and were conducted until the animal made at least 18 switches per 30 min (typically, three to four sessions).

After that, the auto-titration schedule training sessions began. These 35-min training sessions were similar to the side-switching training sessions described above with one notable exception. For every fifth response emitted at the ‘‘stimulation’’ side, the stimulation current intensity was decreased by 2%. A single response on the ‘‘reset’’ side could return the stimulation intensity back to the initial level. The current intensity at which the ‘‘reset’’ response occurred was taken as the threshold stimulation intensity

(provided that the "reset" response was preceded by at least five "stimulation" responses). The individual self-titrated thresholds usually stabilized within 9–14 sessions (less than ±10% variation in threshold across three consecutive days).

The procedure for solvent testing was as follows. During each session, the animal was given an initial 5-min warm-up period followed by a 10-min baseline period. Then, the animal was removed from the chamber and the solvent or solvent mixture was introduced into the exposure box for evaporation. After the evaporation was complete (typically within 10–20 min), the animal was returned to the chamber and the 60-min test session was started.

Each animal was tested repeatedly with different solvents/concentrations and the order of tests was based on a modified Latin Square design ($n$=5 for each solvent concentration level). Tests were conducted twice a week (Wednesdays and Saturdays) provided that the criteria for stable responding were met on the two most recent self-stimulation sessions. Because of the marked response rate decrements at high concentrations for different solvents (presumably due to behavioral toxicity), threshold current intensity levels could not be assessed for all time points or with the complete set of animals per group.

### 2.7. Histology

At the end of the experiment, the rats were euthanized by introducing them into the atmosphere with high $CO_2$ concentration. Rats were decapitated and their brains were quickly removed and stored in 4% formalin. The stimulation site at the end of the electrode tract was examined under a light microscope in cresyl violet-stained sections of 50 μm thickness.

### 2.8. Statistics

In Experiment 1, the primary recorded variable was the number of lever-press responses made by animals during each consecutive 1-min interval providing a measure of rates of responding as a function of successively higher current intensities. In addition, to reduce between-subject variability for statistical purposes, the dependent variable was the difference between response rates before and after solvent exposure for each consecutive 1-min interval.

In Experiment 2, mean response rates on the "stimulation" side and mean stimulation thresholds were calculated separately for six 10-min intervals of the 60-min test,

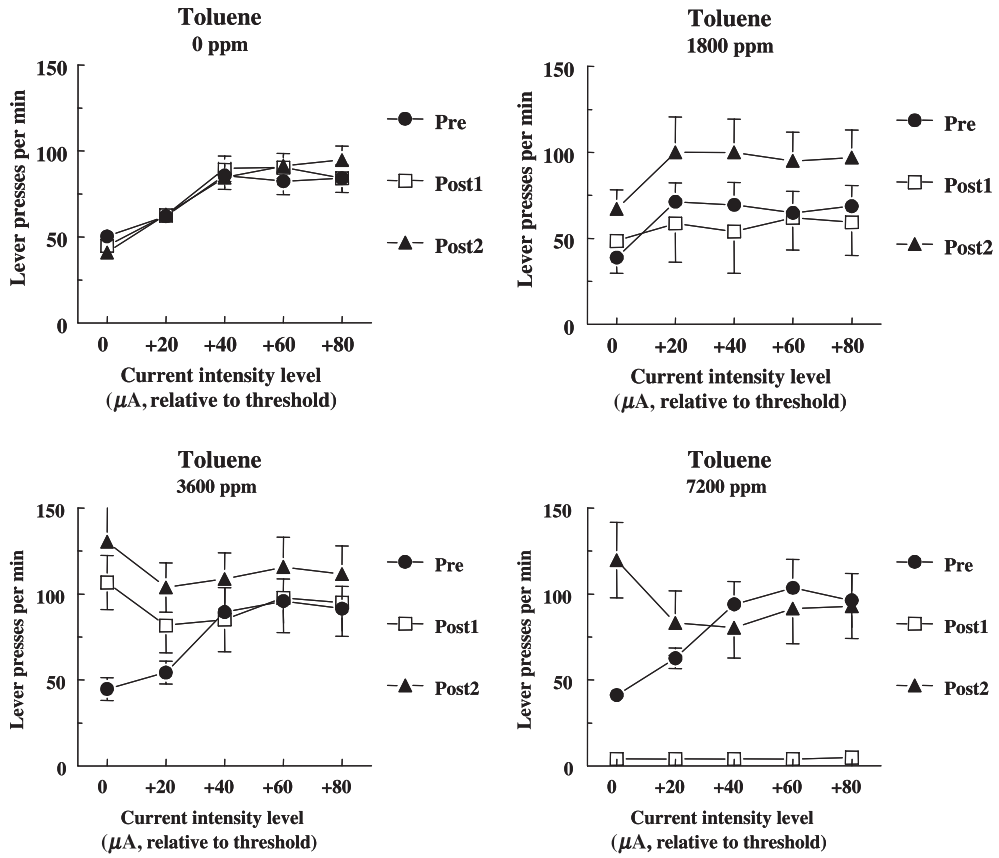


Fig. 1. Effects of toluene on rates of responding for intracranial self-stimulation . Rats were implanted with electrodes into the medial forebrain bundle (MFB) and trained to lever press to receive electrical stimulation under a continuous reinforcement schedule. Rats were exposed to one of four concentrations of toluene (0, 1800, 3600, 7200 ppm) for one hour. Self-stimulation tests were held before (Pre), 1 min after (Post1) and 30 min after (Post2) the toluene exposure. Each test was comprised of five 1-min periods with current intensity levels set at: the threshold level (marked as '0'), 20 μA, 40 μA, 60 μA, and 80 μA above the threshold. Ordinate—total number of lever presses per min. $n$=6 for each data point.

as well as for the 10-min baseline period that always preceded the exposure. To reduce between-subject variability for statistical purposes, the main dependent variables were the response rates and the stimulation thresholds expressed as percentage relative to the baseline. In addition, there was a "perseveration" ratio calculated to control for nonspecific changes in the self-titrated thresholds. The perseveration ratio was calculated as the total number of responses on the "reset" side divided by the total number of "resets." Thus, the perseveration ratio could not be lower than 1 and, when significantly higher than 1, reflected excessive, repetitive behavior on the 'reset' side.

Data were analyzed using SAS-STAT software (ver. 6.11, SAS Institute, Cary, NC). Analysis of the descriptive statistics produced by the SAS-STAT UNIVARIATE procedure demonstrated that some of the data were not distributed normally (Wilks–Shapiro's test). Thus, data were subjected to the distribution-free one- and two-factorial analysis of variance (ANOVA) with repeated measures using a combination of the Rank and General Linear Model (GLM) procedures (SAS Institute, 1990). Briefly, data were ranked and the ranks were later subjected to ANOVA to assess main effects of solvent concentration, current intensity level, and time. Dunnett's test was used for between-group pairwise comparisons (only when indicated by ANOVA results).

## 3. Results

### 3.1. Experiment 1: rate–intensity protocol

Toluene effects on absolute rates of responding (expressed as number of lever presses per minute) are shown in Fig. 1. Under baseline conditions (pretests), rates of lever pressing showed a clear dependence on the intensity of stimulating current, with highest rates occurring at intensities 20–40 μA above the threshold currents. It should also be noted that the animals' behavior was stable as tests conducted 1 or 30 min after the air exposures were not revealing any significant differences in comparison to the pre-exposure test (Fig. 1, upper left panel).

Exposure to toluene produced biphasic effects on response rate (Fig. 1). A lower concentration of toluene (3600 ppm) increased rates of self-stimulation behavior while the higher concentration (7200 ppm) impaired operant performance when the test was conducted 1 min after the exposure. This biphasic effect is also seen in Fig. 2 (left panels), which shows mean difference scores from the pretests to the 1- and 30-min posttests. Importantly, even at this level of toluene exposure, significant increases in rates of lever pressing were observed during the second test (30 min post-exposure). Higher doses of toluene could not

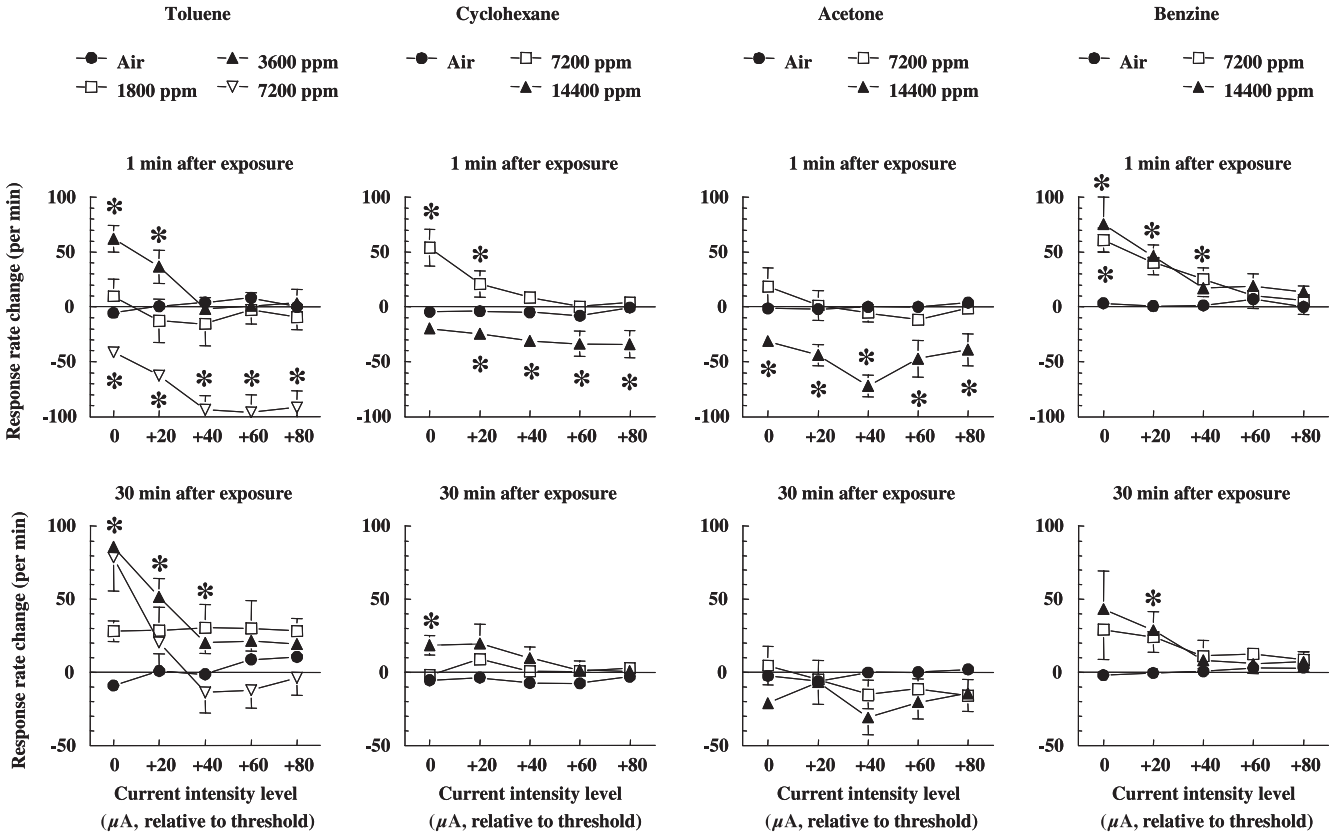


Fig. 2. Changes in rates of intracranial self-stimulation responding as a result of exposure to toluene, cyclohexane, acetone, and benzine. Ordinate—response rate change (relative to the pre-exposure performance, lever presses per minute) after the exposure to various concentrations of the solvents (see Fig. 1 and the text for other details). *$P$<.05 (Dunnett's test), compared to control group (atmospheric air exposure). $n$=6 for each data point.

be tested because of the severe behavioral toxicity reducing the response rate to near-zero levels even 30 min post-exposure.

Two-way ANOVA carried out on the difference scores shown in Fig. 2 confirmed the significant main effect of toluene [1-min post-exposure: $F(3,179)=26.8$, $P<.01$; 30-min post-exposure: $F(3,179)=6.6$, $P<.01$]. In addition, there was found a highly significant interaction between toluene concentration and current intensity factors [1-min post-exposure: $F(12,119)=7.0$, $P<.01$; 30-min post-exposure: $F(12,179)=9.2$, $P<.01$]. Taken together with the visual inspection of the figures and the results of the post hoc individual comparisons, these interactions can be interpreted as toluene producing more facilitation at lower stimulation current intensities.

Exposure to cyclohexane produced effects somewhat similar to those described above for toluene (Fig. 2, second set of panels). At 1 min after exposure, 7200 ppm cyclohexane produced significant increases in rates of responding at the lower current intensities while 14,400 ppm produced significant decreases in rates. At 30 min after exposure, the effects of cyclohexane had almost completely gone, with only the high concentration producing a rate increase similar to what was observed at the lower concentration right after the exposure. These conclusions are supported by the ANOVA showing a main effect of cyclohexane concentration [1-min post-exposure: $F(2,119)=18.7$, $P<.01$; 30-min post-exposure: $F(2,119)=6.7$, $P<.01$]. Significant interactions between concentration and current intensity factors were obtained at 1-min post-exposure [$F(8,119)=5.1$, $P<0.01$] but not at 30-min post-exposure [$F(8,119)=1.3$].

As it is shown in Fig. 2, acetone produced a different pattern of effects. Neither concentration of acetone produced increases at any of the current intensities. At the higher concentration (14,400 ppm), acetone produced a significant impairment of the lever-press responding that was not long-lasting, being significant at 1-min post-exposure [$F(2,119)=22.5$, $P<.01$], but not at 30-min post-exposure [$F(2,119)=3.1$, $P=.07$].

Exposures to benzine facilitated lever pressing at both concentrations tested [Fig. 2; 1-min post-exposure: $F(2,119)=10.2$, $P<.01$; 30-min post-exposure: $F(2,119)=4.8$, $P<.05$]. These activating effects were observed mainly at lower current intensities during the first post-exposure test [benzine concentration by current intensity interaction: 1-min post-exposure: $F(8,119)=5.4$, $P<.01$; 30-min post-exposure: $F(8,119)=1.8$]. No concentration of benzine produced decreases in rates of responding.

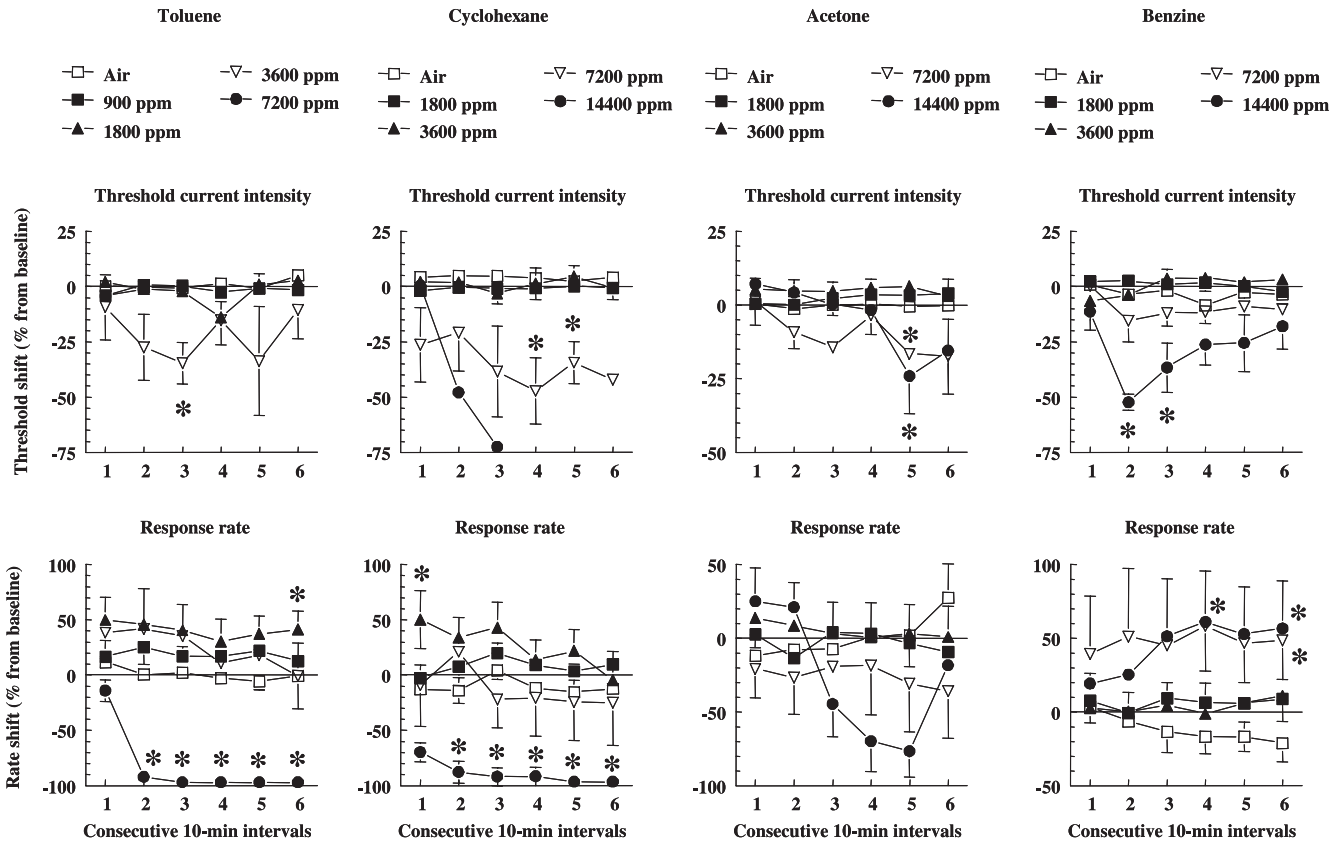


Fig. 3. Effects of toluene, cyclohexane, acetone, and benzine on current thresholds for intracranial self-stimulation behavior under an auto-titration procedure. Tests were conducted before (10-min pretest) and during the 60-min exposure to the solvents. Ordinate—self-titrated thresholds (upper panel) and response rates (lower panel) presented as the % change from the pretest level. *$P<.05$ (Dunnett's test), compared to control exposure to atmospheric air. $n=5$ for each data point.

### 3.2. Experiment 2: auto-titration protocol

As shown in Fig. 3, toluene significantly reduced self-stimulation thresholds [$F(4,95)=4.1$, $P<.01$]. This effect was observed only at one of the tested concentrations (3600 ppm). The higher concentration (7200 ppm) did not change the threshold, presumably because of the severe behavioral impairment observed at this exposure level. This explanation is supported by the evidence for greatly reduced response rates at 7200 ppm [Fig. 3, lower panel; $F(4,120)=45.4$, $P<.01$]. It is noteworthy that, even at the high concentration levels, toluene did not significantly affect the rate of perseverative errors [Fig. 4; $F(4,22)=0.1$].

Among the tested solvents, cyclohexane seemed to produce the most robust effects on self-stimulation behavior [stimulation thresholds: $F(4,87)=9.2$, $P<.01$; response rate: $F(4,120)=35.0$, $P<.01$]. At the highest concentration (14,400 ppm), animals stopped responding soon after the exposure had begun. As a result, stimulation threshold data were collected only for the first half of the session (Fig. 3). At the concentration of 7200 ppm, cyclohexane did not significantly reduce response rate but there was still a substantial reduction in stimulation thresholds. Meanwhile, at 7200 and 14,400 ppm, there was also a marked increase in the rate of responding on the 'reset' side [i.e. increase in the perseveration ratio; Fig. 4; $F(4,22)=11.8$, $P<.01$].

Effects of acetone did not develop until the fourth or fifth 10-min interval when a significant reduction in the self-stimulation thresholds was observed [Fig. 3; $F(4,101)=7.1$, $P<.01$]. There was no significant facilitation of response rate at any of the concentrations tested while, at the higher concentrations, acetone decreased the rates of lever pressing [$F(4,120)=3.4$, $P<.05$]. Performance at the 'reset' side (perseveration ratio) was slightly increased after the exposure to the higher concentrations of acetone [Fig. 4; $F(4,24)=3.1$, $P<.05$].

Unlike other tested solvents, benzine, even at high concentrations, did not suppress rates of responding while the self-titrated stimulation thresholds were decreased in a concentration-dependent manner (Fig. 3). It should be noted that the reduction in stimulation thresholds [$F(4,120)=20.6$, $P<.01$] by benzine was accompanied by increased rates of responding on the 'stimulation' side [$F(4,120)=10.5$, $P<.01$]. Importantly, benzine did not affect the performance at the 'reset' side [Fig. 4; $F(4,24)=2.0$].

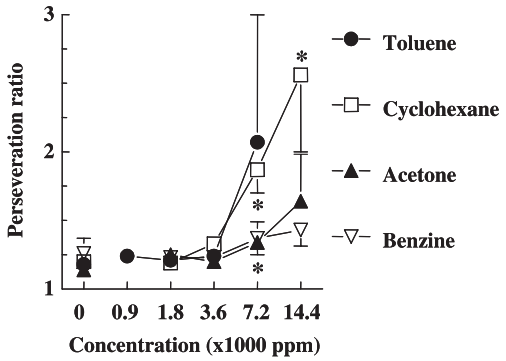


Fig. 4. Effects of toluene, cyclohexane, acetone, and benzine on intracranial self-stimulation: perseveration ratio (see the text for details). *$P<.05$ (Dunnett's test), compared to control exposure to atmospheric air. $n=5$ for each data point.

## 4. Discussion

The present experiments were designed to analyze the effects of several solvents on electrical brain self-stimulation behavior. In agreement with the previous studies (Yavich and Zvartau, 1994), tested solvents were generally found to facilitate the self-stimulation behavior, although there were some interesting differences among the materials tested.

In the first set of experiments, the design of the Yavich and Zvartau (1994) study was reproduced and the results were confirmed and extended. Toluene and benzine significantly and in a concentration-dependent manner increased the rates of self-stimulation behavior. Cyclohexane had a short-lasting effect at a single concentration level (7200 ppm). Just like for the other solvents, exposures to the higher concentrations were impairing the operant performance and could hardly be tested. However, it is still noteworthy that even at the highest concentration (14,400 ppm) rate increasing effects of cyclohexane could be revealed after a 30-min recovery period. In contrast, acetone had no significant facilitating effects but rather suppressed operant responding at the high concentration immediately after the exposure as well as 30 min later. In these experiments, each solvent was tested across a very limited range of concentrations. These concentrations were selected largely based on the earlier published reports (Yavich and Zvartau, 1994). The purpose of these tests was mainly to replicate the previous data and to assess the behavioral toxicity levels for the tested compounds. Nevertheless, one should note that even in the second set of experiments (self-titration experiments discussed below) lower concentrations of acetone (1800 and 3600 ppm) had no significant effects on self-stimulation behavior.

Toluene, benzine, and cyclohexane affected mostly responding at the lower levels of stimulation current (i.e. early in the test session). It may be argued that, after the animals are taken from the exposure chambers, there is some residual solvent remaining in their body and its clearance parallels the disappearance of the facilitating effects on behavior. However, for all three solvents, such interpretation can be ruled out since similar current intensity-dependent facilitating effects were observed 30 min after the exposure. Thus, it is more likely that the explanation may come from the general analysis of the stimulation intensity–response rate relationships. These increases only at low current intensities may have been a reflection of ceiling effects at the higher intensities. Under control conditions (Fig. 1, upper left panel), increases in response rate

occurred as current intensities were increased to 20 and 40 μA above the threshold, but further current increases were not associated with further increases in rates. Thus, it may be that limitations in the ability to respond faster or maximization of the reinforcing efficacy at intermediate intensities precluded enhancement by solvents.

All in all, toluene and benzine exerted clear-cut response rate-facilitating effects. Cyclohexane did too, but they were shorter lived. Acetone was clearly without facilitating effects under these conditions. To further clarify these differences among solvents and to test whether these rate-facilitating effects reflected solvent exposure-induced sensitization of brain reward systems, a second study was performed. This experiment utilized a response-rate free measure of sensitivity to electrical brain stimulation reward. Indeed, self-stimulation protocols may provide misleading information if the self-stimulation behavior is assessed only using the rate-dependent measures (Liebman, 1983; Schaefer and Michael, 1992). Also, it should be noted that these initial experiments were evaluating the effects of solvent exposures outside the exposure chambers (i.e. after the exposure). Therefore, one may argue that the rapid recovery of operant performance after the solvent exposure is terminated could underestimate solvent effects during exposures. In addition, tests after removal from the exposure chamber may result in behavioral contrast. In other words, the rate-increasing effects observed post-exposure may be related to the aversive effects of solvent exposure rather to their potential abuse properties.

In the second set of experiments, the major dependent variable was the self-titrated threshold of stimulation current intensity measured while the animals were exposed to the solvents. These stimulation thresholds were monitored before and during the exposure to the solvents or to the air and were found to be fairly stable for each individual rat. It needs to be emphasized that the operant responding at both 'stimulation' and 'reset' sides was very stable and was routinely monitored throughout the study by conducting multiple air exposure tests.

All tested solvents were found to reduce self-stimulation thresholds and in nearly every case the effect was very robust. However, there were significant differences between the solvents with regard to the nature and selectivity of these threshold-decreasing effects.

Toluene and benzine decreased the self-stimulation current intensity thresholds while increasing the rates of responding. These solvents did not affect the responding at the 'reset' side. Therefore, effects of these solvents may be regarded as a rather specific increase in the rewarding value of electrical stimulation. In contrast, cyclohexane and acetone markedly decreased the self-titrated stimulation thresholds only at doses that suppressed overall response rates. Perhaps even more important, unlike toluene and benzine, effects of cyclohexane and acetone were accompanied by the significant increase in the 'perseveration' ratio. This ratio was calculated to control for the nonspecific changes in the self-titrated thresholds and was expressed as the total number of responses on the "reset" side divided by the total number of "switches." Thus, "perfect" behavior would be characterized by the perseveration ratios equal or just above 1. When significantly higher than 1, this ratio indicates excessive, stereotypy-like behavior on the 'reset' side. This is exactly what was observed during the exposures to cyclohexane and acetone. Animals' motor performance was greatly impaired (as partially reflected by the reduced response rates) and they would often respond repeatedly at the same side until making an effort to switch to another side. One consequence of this behavior was that the stimulation thresholds appeared to be reduced. Another consequence was the increased perseveration ratio. These effects of cyclohexane and acetone can also be viewed as a marker of behavioral toxicity possibly relating to the impaired discrimination between the stimulation and reset sides. To the best of our knowledge, there are no published reports to confirm or challenge this explanation. However, in this study, toluene did not affect the 'perseveration' ratio and it was earlier reported that toluene does not affect discriminative behavior up to the concentrations of 5400 ppm (Rees et al., 1987b).

Taken together, the results from both studies allow a firm conclusion to be made that toluene and the solvent mixture benzine can facilitate electrical self-stimulation behavior, as evidenced by both increases in rates of responding and in selective lowering of current reward thresholds. In this respect, these solvents produce effects similar to that produced by drugs of abuse such as cocaine-like stimulants and opiates (Kornetsky and Bain, 1992). These results with toluene are entirely consistent with a mounting body of evidence that it has abuse-related behavioral effects in other animal models (Balster, 1991; Evans and Balster, 1991), including direct reinforcing effects in self-administration studies in monkeys (Weiss et al., 1979; Wood, 1978; Yanagita et al., 1970) and mice (Blokhina et al., 2001). Thus, toluene continues to be a good candidate for comparative studies of abused solvents.

Thus, the present study suggests that toluene may sensitize dopaminergic mesocorticolimbic pathway to electrical stimulation. Dopamine has long been implicated in the rewarding effects of various abused drugs most of which share the ability to enhance dopamine metabolism in the mesocorticolimbic areas (Wise, 2002). Importantly, toluene was also shown to enhance dopamine metabolism although this effect was limited to the prefrontal cortex and was not observed in the ventral striatum unless toluene was administered in combination with cocaine (Gerasimov et al., 2002).

Toluene is among the most commonly abused inhalants and mechanisms of its effects receive a lot of attention. For instance, toluene enhances $GABA_A$ receptor function (Beckstead et al., 2000) and inhibits NMDA receptor-mediated responses (Cruz et al., 2000). Accordingly, in drug discrimination studies, toluene was reported to substi-

tute for several GABAergic drugs such as pentobarbital and ethanol (but not diazepam) as well as for NMDA receptor antagonist phencyclidine (Bowen et al., 1999; Rees et al., 1985, 1987b). Furthermore, toluene produces anxiolytic effects (Lopez-Rubalcava et al., 2000; Wood et al., 1984) which are also commonly reported for both GABA receptor agonists and NMDA receptor antagonists (e.g. Bourin and Hascoet, 2001; Wiley, 1997). Thus, since both GABA agonists and NMDA receptor antagonists are capable of facilitating the brain stimulation reward (e.g. Seeger et al., 1981; Tzschentke and Schmidt, 1999), it is likely that the effects of toluene observed in the present study have both GABA- and glutamatergic mechanisms.

Solvent mixtures such as benzine have not been very systematically studied in behavioral research. One reason for this is the wide variation in the composition of these mixtures. Nonetheless, solvent mixtures, such as benzine, are very commonly available and abused, so it is important to show that they too can be studied just as the individual components. It is important to note that the toluene-like effects seen here with this mixture may not occur with all mixtures, even other mixtures of the highly volatile low boiling point fractions of petroleum.

A previous self-stimulation study with the commercial solvent mixture revealed a rightward shift of the current–response curve indicative of increased threshold current intensity (Yavich et al., 1994). In addition to toluene and benzine, this solvent mixture also included ethyl acetate and methylene chloride, which could have effects of their own overriding the facilitatory effects of toluene and benzine that were revealed in the present study. Studies of the individual components of these mixtures can help determine the basis for their acute effects.

Cyclohexane produced many effects that were similar to toluene. It increased response rates at low current intensities and lowered current thresholds, but the latter effect was not as selective as was seen with toluene (i.e. it was accompanied by response rate decreases and perseverative errors). The effects of cyclohexane were clearly much short-lived than those of toluene, as evidenced by the rapid recovery seen in the first experiment. Not enough is known about the profile of effects of cyclohexane to conclude with any certainty that the differences seen between it and toluene in the present experiments reflect meaningful differences in the nature of their acute effects or in their abuse potential.

A similar conclusion can be made for acetone, although its effects were the most different from those of toluene under the present testing conditions. Acetone did not result in increased rates of responding at any concentration and, while it did decrease reward thresholds, its effects may have been very nonselective, since decreases in rates of responding and increases in perseverative responding also occurred. Despite its widespread use, acetone too has not been well characterized in behavioral research. Tentatively, it appears that acetone may not have a clear separation of abuse-related effects and direct toxic effects on behavior as does toluene. The implications of this for acetone abuse await further investigation. It may be that, as profiles of effects of specific solvents become better understood, some subclassifications among them may be possible based on animal studies of this type.

In conclusion, the results of these studies confirm the value of using electrical self-stimulation behavior to study abuse-related effects of abused solvents. We have also shown that it is possible to study the effects of solvents both during and after exposures. The evidence for abused drug-like effects of toluene and benzine suggests that this model may also have value in creating profiles of individual solvents that may be useful in predicting their abuse potential. We have also shown that testing procedures that allow for a response-rate free assessment of reward thresholds may be important for studying solvents. It is likely that solvents that selectively facilitate self-stimulation reward will be more likely to be abused. This model also has promise for studying brain mechanisms for solvent abuse and for comparing profiles of effects of individual solvents.

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