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CONDITIONED PLACE PREFERENCE TO ACETONE INHALATION AND THE EFFECTS ON LOCOMOTOR BEHAVIOR AND ^{18}FDG UPTAKE

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ABSTRACT

Acetone is a component in many inhalants that have been widely abused. While other solvents have addictive potential, such as toluene, it is unclear whether acetone alone contains addictive properties. The locomotor, relative glucose metabolism and abusive effects of acetone inhalation were studied in animals using the conditioned place preference (CPP) paradigm and [^{18}F]2-fluorodeoxy-D-glucose (^{18}FDG) imaging. The CPP apparatus contains two distinct conditioning chambers and a middle adaptation chamber, each lined with photocells to monitor locomotor activity. Adolescent Sprague-Dawley rats (n=16; 90-110 g) were paired with acetone in least preferred conditioning chamber, determined on the pretest day. The animals were exposed to a 10,000 ppm dose for an hour, alternating days with air. A CPP test was conducted after the 3rd, 6th and 12th pairing. In these same animals, the relative glucose metabolism effects were determined using positron emission tomography (PET) imaging with ^{18}FDG. Following the 3rd pairing, there was a significant aversion to the acetone paired chamber (190.9 ± 13.7 sec and 241.7 ± 16.9 sec, acetone and air, respectively). After the 6th pairing, there was no significant preference observed with equal time spent in each chamber (222 ± 21 sec and 207 ± 20 sec, acetone and air-paired, respectively). A similar trend was observed after the 12th pairing (213 ± 21 sec and 221 ± 22 sec, acetone and air-paired, respectively). Locomotor analysis indicated a significant decrease ($p<0.05$) from air pairings to acetone pairings on the first and sixth pairings. The observed locomotor activity was characteristic of central nervous system (CNS) depressants, without showing clear abusive effects in this CPP model. In these studies, acetone vapors were not as reinforcing as other solvents, shown by overall lack of preference for the acetone paired side of the chamber. PET imaging indicated a regionally specific distribution of ^{18}FDG uptake following acetone exposure. Further studies using different concentrations are required to better understand the locomotor and behavioral effects of acetone. This study confirms that the combination of microPET and the CPP paradigm can be used to elucidate the effects of abused solvents vs. non-abused solvents in inhalants.

INTRODUCTION

Inhalant abuse has become an increasing problem in the United States and around the world, a major problem especially among children and adolescents [1,2] even though it does not carry the same publicity as illegal drug use. Inhalants, due to the inexpensiveness and unrestricted availability, frequently become the first substance abused by children. While most teenagers cease to abuse inhalants, many progress to drugs with larger consequences, including marijuana, cocaine and opiates, or even using them alongside inhalants [3,4]. As a result, it becomes important to determine which inhalants carry the greatest risk of abuse.

Commonly abused products, such as paints, varnishes, paint thinners and adhesives, consist of many solvent components. In

the literature, toluene is known to be a major component that causes abusive effects in inhalants in a dose dependent manner [5]. Solvents such as acetone have not been as extensively covered in literature. Acetone, commonly found in some paint thinners and nail polish, has high water solubility. This causes the acetone to diffuse rapidly into the blood while entering the brain at a much slower rate than solvents such as toluene. As a result it creates central nervous system (CNS) effects that are not as strong but have a much longer presence in the system [6]. It seems unlikely that solvent abusers would select acetone because of its slow onset and prolonged duration of action however, preliminary studies indicate a preference which is why we plan to study it further.

Conditioned Place Preference (CPP) is an indirect model of drug seeking behavior. Focusing on environments previously associated with a drug, it is used to measure the motivational effects of inhalants rather than the actual act of administering [7]. We will use the CPP paradigm to test the hypothesis that acetone is reinforcing to laboratory animals. We will also test the secondary hypothesis that acetone, like other CNS depressants, alters locomotor activity [6, 8]. Our unique conditioning chamber is equipped with automated photocells capable of measuring locomotor activity simultaneously as conditioning occurs and will allow us to assess acetone's role in CNS depression shown in other abused solvents [9]. Determine whether acetone produces locomotor sensitization (increase response to drug) or tolerance (decrease response to drug), two measures of altered CNS functions studied with drugs of abuse [10,11].

In addition to measuring locomotor behavior and place preference, the neurochemical effects of acetone will be studied using microPET (positron emission tomography). PET studies can be used to measure the acute neurochemical responses to an abused drug or track the addictive drug's path in the brain [12,13]. Neuroimaging studies with 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG), an analog of glucose, allow measurement of relative glucose metabolism, more importantly local cerebral metabolism [14]. PET allows us to study drug effects and how they contribute to reward and reinforcement. Pet can also be used to investigate the impact drug abuse has on the system [13]. Information from this study will affect the attention given to inhalants, prevention efforts currently in process as well as pharmacologic treatment strategies.

MATERIALS AND METHODS

Subjects

Adolescent Sprague-Dawley rats (n=16; Taconic Farms, Germantown, NY) measuring 90-110 grams at the beginning of the study were used. The rats were housed randomly in pairs and kept on a 7am/7pm light cycle and given free access to food and water. All testing and conditioning of the animals were preformed daily at 9am. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee.

Conditioned Place Preference Apparatus

The conditioned place preference apparatus (ENV-013 MED Associates Inc.) consists of two conditioning chambers (21×21×27.5 cm) separated by a middle chamber with access to each chambers facilitated by two guillotine doors. One conditioning chambers contains black walls with a white smooth floor, the other contains white walls with a steel mesh floor. The middle adaptation chamber has gray walls and a smooth floor. Conditioning chambers contain clear plexiglass ceilings that becomes airtight when shut. Infrared photocells line the walls of each chamber to monitor locomotor activity and time spent in each chamber. The photocells, positioned at the level of the animal's head, are connected to MED-PC for Windows and Delphi TM4 and programmed to measure beam breaks when the animal enters the chamber (head and the front torso).

In order to maintain the equilibrium concentration of acetone vapor in the conditioning chambers. Each of the conditioning chambers contains an opening at the far top end that delivers the inhalant vapors and along the bottom to allow stabilization of atmospheric pressure. Before the initial exposure to the vapors, concentration equilibration required for 20 minutes.

Acetone Administration

Acetone (99%) was purchased from Sigma-Aldrich (Milwaukee, WI). Mixtures of acetone vapors and air were metered using two mass flow controllers (Dyna-Blender by Matheson Tri-Gas, Mongomerville, PA) with a total flow of gas mixture set at 2 L/min. During conditioning, the acetone was kept at 0°C and bubbled to retard evaporation (1.75 L/min of pure air and 0.25 L/min of acetone) for a concentration of 10,000 ppm (calibration on a gas chromatograph). During conditioning, rats were exposed to the acetone vapors or air at 24 hours intervals for an hour duration.

CPP Procedure

Preconditioning phase: The animals were transported between the Brookhaven Laboratory Animal Facility and the Chemistry department and handled to adjust them to the transportation procedure and conditioning environment for three days.

Pretest day: Each rat was placed into the middle chamber for 5 minutes of adaptation. The animals were allowed to freely explore the three chambers for 15 min. The time spent in each chamber was recorded.

Conditioning phase: For the first group of animals (n=8), a CPP test was conducted following the 6th and 12th pairing. For the second group of animals (n=8), a CPP test was conducted after 3 pairings. The pairings (one acetone and air dose) were administered on consecutive days, including weekends, alternating the doses of acetone and air. The rats were assigned acetone in the side they had least preferred on the pretest day. During conditioning, noise was brought to the minimum and presence of humans were restricted.

Test day: 24 hours after last acetone administration, the animals were tested for a place preference. Animals were placed into the middle adaptation chamber for 1.5 minutes (since animals have now habituated to the chamber) and then allowed to freely explore the three chambers for 15 minutes. The time was recorded as well as the number of chamber crossings that occurred during the test.

MicroPET Imaging

Before animals were imaged, an additional pairing was administered. The animals received an intraperitoneal (IP) injection of ^{18}FDG (~ 1.0 mCi; $t_{1/2}$ 110 min) for a 45-minute uptake in their home cage and scanned for 10 or 20 minutes. MicroPET images were taken using an R4 tomograph (Concorde Microsystems, Knoxville, TN). Following the scan, a plasma glucose value was measured (standard glucose testing strips) from the lateral tail vein. MicroPET images were analyzed by spatial pre-processing (realignment, coregistration and normalization) with SPM2 (Statisical Parametric Mapping 2) and dose corrected. A ROI (region of interest) template was applied and analyzed using the PMOD (Pixel-wise Modeling tool 2.65) software package.

Statistical Analysis

A student's T test (1-tailed, 2-variance) was used to determine significance ($p < 0.05$). Determination of locomotor activity was defined as the break of two consecutive beams. Locomotor analysis was analyzed using area under the curve with SigmaPlot (9.0).

RESULTS

Conditioned Place Preference

At the pretest, the first group of animals (n=8) spent 215.4 ± 32.11 sec and 197.6 ± 28.7 sec (mean ± standard deviation) in the white and black chambers, respectively. Following 6 pairings of acetone and air exposure, the animals did not exhibit a significant preference for either the acetone paired side (221.7 ± 20.6 sec) or the air paired side (206.9 ± 20.0 sec). After the 12th pairing (Figure 1), these same animals as a group did not show a significant preference

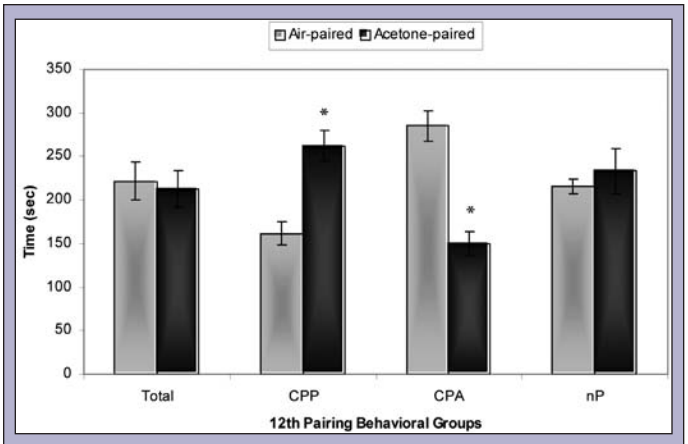

Figure 1. CPP results after the 12th pairing with the preference of the animals as a group. The three distinct groups were separated out into CPP (n=3), CPA (n=3) and nP (n=2).

toward either conditioning chamber (212.7 ± 21.1 sec and 221.4 ± 21.6 sec, acetone paired and air paired chamber, respectively). Three animals out of this group expressed a positive place preference ($p < 0.006$) with 262.0 ± 17.5 sec in the acetone-paired chamber and 161.3 ± 13.3 sec in the air-paired chamber. Three animals expressed a significant ($p < 0.002$) conditioned place aversion (CPA) with 149.6.4 ± 13.6 sec in the acetone paired side and 285.2 ± 17.3 sec in the air paired side. Two animals expressed no preference (nP) for either chamber (233.5 ± 25.8 sec and 216 ± 8.3 sec; acetone and air paired chamber, respectively).

A separate group of animals (n=8) at the pretest had no preference for either side of the conditioning chamber spending 217.1 ± 19.1 sec and 176.3 ± 32.9 sec in the white and black chamber, respectively. Following the 3rd pairing, there was a significant ($p < 0.02$) aversion for the acetone paired side of the chamber with the animals spending 190.9 ± 13.7 seconds in the acetone paired side vs. 241.7 ± 16.9 seconds in the air paired side. Figure 1 illustrates the preference for the behavioral groups after the 12th pairing.

Locomotor Activity

Figure 2 illustrates the average locomotor activity comparison per minute between acetone and air showed a significant ($p<0.01$)

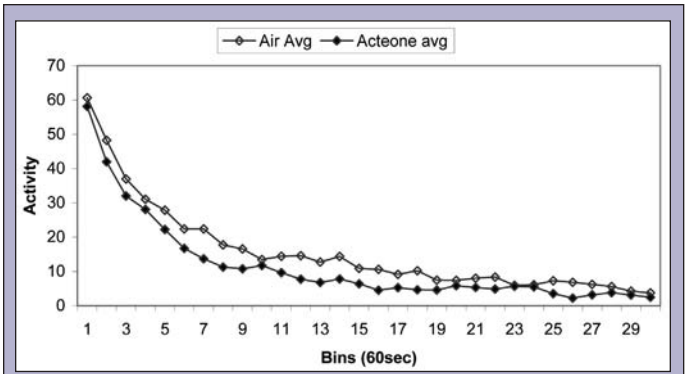

Figure 2. Locomotor activity during each initial 30 minutes of air and acetone conditioning days, average of all the pairings days combined.

decrease during the first 30 minutes of conditioning from the air exposed days to the acetone exposed day in the same pairing. Standard deviations of the curves fell in the range of 0-2, therefore not displayed on the figure. During the initial first 5 minutes of the locomotor activity the respective slopes for the air and acetone were –6.7009 and –7.8685 (set at an intercept of 60) with a marked difference of 18% between the two exposures.

During the hour conditioning period, there was a gradual decrease in the locomotor activity throughout the initial six pairings with the exception of the 3rd pairing, revealing significantly lower acetone locomotor activity than on the air exposure day, shown in Figure 3. From the first pairing to the sixth pairing, there was a decrease in the ratio of acetone exposed locomotor activity to air exposed locomotor activity. For the first six pairings the average ratio was 69.2%. During the final six pairings there was a fluctuation in the locomotor activity with no pattern displayed. The animals no longer displayed a significant difference between the acetone and air activity, though acetone is still lower than the air. For the final six pairings the average ratio of the acetone to air exposures of each pairing was 83.6%.

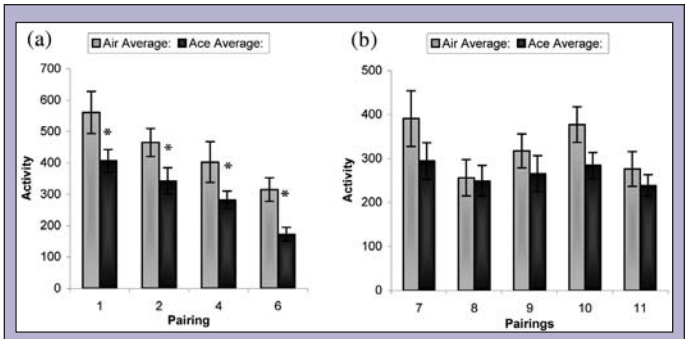

Figure 3. 1, 2, 4, and 6th pairing in the initial six pairings: (a) the first five pairings (b) final six pairings of exposure. Values were determined using an area under the curve for the first 20 minutes during each conditioning period.

FDG Neuroimaging

The baseline images (Figure 4) for which all acetone exposed animals were compared to were taken from other animals that were

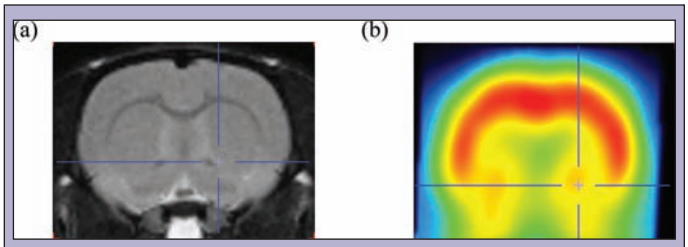

Figure 4. Baseline Images: (a) MRI map of the coronal plane of a rat brain (b) image of the FDG uptake in the frontal cortex and striatum.

of the same group, age, and weight when they arrived. Images of the animals after the 12th baseline (Figure 5), displaying the ^{18}FDG uptake for each of the behavioral groups are on the top row, along the bottom row the images are overlapped onto an MRI scan to distinguish regions of the brain, specifically the striatum and cortex.

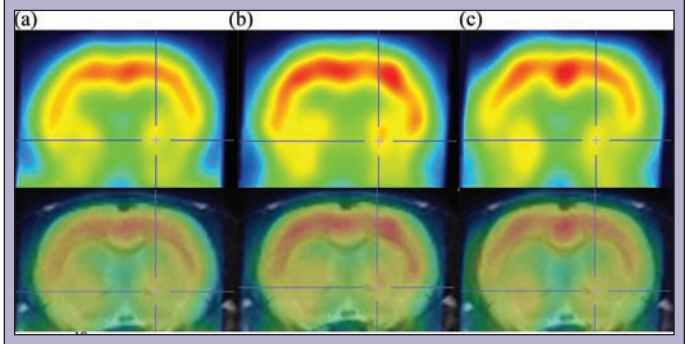

Figure 5. ^{18}FDG uptake following chronic exposure of acetone on the relative brain metabolism for the (a) conditioned place preference (CPP); (b) conditioned place aversion (CPA); and (c) no preference (nP) behavioral groups.

Analysis of the relative glucose metabolism for regional changes to whole brain (Figure 6) showed there were differences in the regional distribution between the three different behavioral groups. Observed

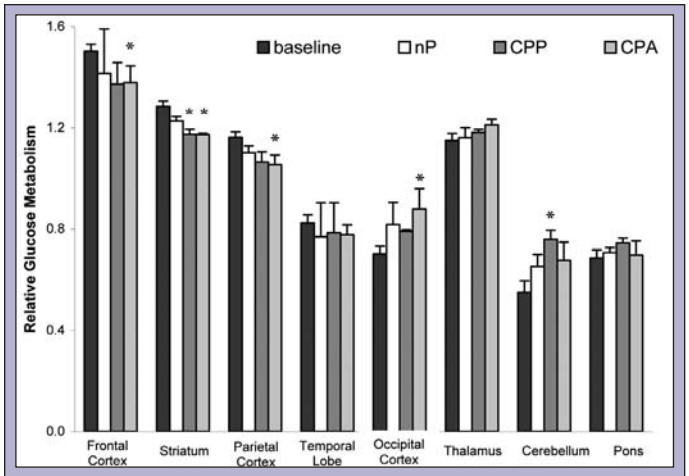

Figure 6. Relative glucose metabolism with regionally specific ^{18}FDG uptake. *Significant differences ($p < 0.05$).

that FDG uptake was regionally specific with decreases in the frontal cortex, striatum, parietal cortex and temporal coretex while there were increases in the occipital cortex, thalamus, cerebellum and pons. The CPA group displayed a significant ($p < 0.05$) difference from the baseline in the Frontal Cortex, the Striatum, the Parietal Cortex and the Occipital Cortex, CPP group: the striatum and cerebellum were the regions that displayed a significant difference. Results analyzed through percent injected dose/cc (Figure 7) indicated only CPA behavioral group had a significant difference from the baseline.

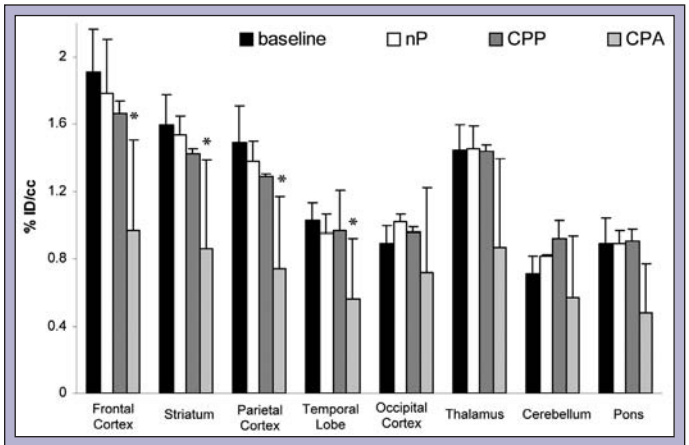

Figure 7. Percent injected dose/cc of brain tissue. *Significant decreases ($p < 0.05$) in the frontal cortex, striatum, parietal cortex and temporal lobe in the CPA behavioral group.

DISCUSSION

The lasting behavioral effect of abused drugs is an extremely important question relating to drug addiction research. The animal model for abuse liability of solvents has been very successful for toluene. Nevertheless, the abuse potential of other solvents common to household products remains to be established. We therefore extend out previous method for determining the abuse liability of toluene [5,15] to acetone, a common ingredient in products such as fingernail polish remover. Based on our previous data and data

from biochemical studies [6,16] reinforcing doses of acetone should be roughly five times higher than reinforcing doses of toluene. Our studies indicate the optimal reinforcing dose of toluene as 2,000 ppm, so therefore we chose a dose of 10,000 ppm acetone, since acetone is much less potent that toluene for producing intoxicating effects in mice (Bruckner and Peterson). We also obtained parallel measures of locomotor activity and regional changes in brain energy demand. Our overall goal was to obtain a better understanding of the effects that acetone has on locomotor behavior, place preference and metabolic consequences for reinforcing or aversive exposures to acetone.

Our results provides evidence that a dose-dependent CPP could not be obtained with an exposure of 10,000 ppm and a pairing duration of 60 minutes. The "dose" of inhaled solvent incorporates both the concentration during exposure and the duration for which animals are exposed to that concentration. Here, the dose was based on preliminary data which showed that six pairings of 30 minutes, followed by three pairings of 60 minutes, yielded a significant CPP to acetone. Based on this and biochemical data, there are several reasons why this particular 'dose' did not produce a place preference for acetone. First, the reinforcing concentrations of acetone have not been established and it is possible that 10,000 ppm is in excess; second, the metabolic response in acetone-preferring animals differs significantly from the acetone — averse animals; and third, our compiled data would suggest that the 'dose' of acetone may need the incorporation of a third variable: exposure titration. Animals may need to be exposed to smaller doses of acetone prior to being exposed to a concentration that is as reinforcing as toluene.

While there was no preference following the 6^{th} and the 12^{th} pairing, it is significantly important that the animals showed an aversion to the vapor. With the addition of the preliminary data showing a significant preference. Hypothesis on the addictive effects of acetone based on the duration that animals were exposed to the volatile vapors can be formed. The sensitivity of the dose would explain distinct behavioral groups exhibited after the 12^{th} pairing. The hour duration chosen was established by the pharmakinetics of the solvent. Acetone is more water soluble than toluene allowing it to absorb into the blood stream more rapidly but less inclined to diffuse into the brain. Known as an unmetabolized vapor, enzymes are unable to break it down [17,18]. As a result, higher levels of acetone have been found in the blood than in the brain [6,19], creating less potent CNS effects with a gradual but longer duration. It seems that initially the hour doses are too overpowering for the animals and cause the aversive affects. Instead the animals need smaller doses to adjust to the solvent before increasing the doses (or titrating up) to create the preference found with preliminary data.

Analysis of locomotor activity indicates that the solvent carries the characteristics of a CNS depressant producing concentration-dependant narcoses in animals [6]. The significant decrease in average activity on acetone exposure days and air exposure days seems to provide evidence of this characterization [8]. Individual pairings were examined to study if acetone effects became more evident with increasing pairings. These measures demonstrated a marked decrease in the ratio between the acetone and air for the initial 6 pairings, indicating that acetone is producing a behavioral effect on the animals during acute exposure. This supports previous studies that indicated a decrease in locomotor activity following acetone exposure [6,8] and provides evidence that the concentration used in this study was sufficiently high. The return of the acetone locomotor activity to 83% of the acetone and remaining approximately at the level seems to suggest that larger doses of acetone are needed in order for the similar trend of effect to continue. It can be hypothesized that a titration increase could carry the decreasing trend found after the initial six pairings through the final 6 pairings. Our results indicate that reinforcing effects of acetone can not clearly be established, as toluene that has been shown to have large reinforcing properties through the use of the CPP paradigm [5,15]. Unlike studies done on dose-dependent effects with other drugs of abuse [20,21], acetone is unique in that there are numerous various variables that can be manipulated (number of pairings, pairing duration, pairing concentration and pairing titration). Further studies need to be conducted to verify the affect of these variables on preference.

The PET studies provide evidence that acetone contains a differential regional uptake distribution in various regions of the CNS that possibly denote the signs and symptoms that are related to its abuse. The application of ^{18}FDG is key in determining the brain regions that are most sensitive to the effects of the given drug. By analyzing the data, regional to whole brain measures of relative glucose metabolism can be used to identify distinct changes to chronic exposure of acetone. Of these regions, striatum and orbitofrontal cortices have been associated with addiction and reward related behaviors of drugs of abuse [22]. Overall it can be determined that there was a regionally specific distribution in the brain following the acetone exposure. The animals will be imaged two months after the initial scan to determine if there was a return to the baseline or if there were permanent effects of these animals.

Further studies are needed to determine the reinforcing 'dose' of acetone. These data clearly distinguish toluene and acetone in terms of pharmacodynamic effects, consistent with their differing pharmacokinetic profiles. Further, these studies substantiate the hypothesis that not all inhalants produce their intoxicating effects by non-specific mechanisms. Our results with acetone are very different than equipotent doses of toluene, suggesting that each inhalant possesses a unique behavioral and metabolic profile, which may effect its dependence liability. Clearly the reinforcing properties of acetone, and of inhalants as a whole, are far more complex than previously thought.

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