Published in final edited form as:

Behav Pharmacol. 2014 February ; 25(1): 2–11. doi:10.1097/FBP.000000000000017.

Discriminative stimulus effects of nitrous oxide in mice: comparison to volatile hydrocarbons and vapor anesthetics

Kellianne J. Richardson¹ and Keith L. Shelton^{1,2}

¹Department of Pharmacology and Toxicology, Virginia Commonwealth University School of Medicine, P.O. Box 980613, Richmond, Virginia 23298-0613

Abstract

The abuse-related behavioral effects produced by nitrous oxide (N₂O) gas have been suggested to be unique amongst abused inhalants. The drug discrimination paradigm in animals can be used to study subjective effects of drugs in humans and can be used to test this hypothesis. The goals of the present experiment were to establish a nitrous oxide discrimination in mice and compare its discriminative stimulus effects to those of abused volatile vapors and vapor anesthetics. Sixteen B6SJLF1/J mice were trained to discriminate 10 min of exposure to 60% N₂O + 40% oxygen from 100% oxygen. The time course of N₂O discrimination was examined followed by cross-substitution testing with abused vapors, volatile anesthetics, ethanol, D-amphetamine and 2-butanol. Mice acquired the N₂O versus O₂ discrimination in 40 days. N₂O fully substituted for 10 min of exposure to 60% N₂O in a concentration-dependent manner. Full substitution required 7 min of 60% N₂O exposure but the offset of stimulus effects following the cessation of exposure was more rapid. The aromatic hydrocarbon toluene almost fully substituted for N₂O. 1,1,1-trichloroethane (TCE), methoxyflurane, isoflurane and ethanol exhibited lesser degrees of substitution, and D-amphetamine and the odorant 2-butanol did not substitute at all for N₂O. Given the varying degrees of incomplete substitution by test compounds, the discriminative stimulus properties of N₂O and, perhaps, its subjective effects in humans are probably not unique. As none of the inhalants tested fully mimicked N₂O, its overall effects may include one or more novel stimulus components.

Keywords

drug discrimination; nitrous oxide; inhalants; toluene; analgesic; anxiolytic; anesthetic

Introduction

Nitrous oxide (N₂O) an analgesic and anxiolytic gas used routinely in dentistry and medicine is widely abused. Adolescents and medical staff in anesthesiology and dentistry are particularly at risk for abuse of N₂O (Bell et al., 1999; Seidberg and Sullivan, 2004; Substance Abuse and Mental Health Services Administration, 2008). N₂O may appeal to adolescents because it is inexpensive, legal to obtain and easy to conceal. Unlike most inhalants, which are primarily abused by younger adolescents, N₂O is most commonly abused by adolescents aged 16–17 (Substance Abuse and Mental Health Services Administration, 2008). N₂O may appeal to medical professionals because it is short acting, undetectable in drug testing and easily diverted (Seidberg and Sullivan, 2004). Repeated N₂O use can lead to medical complications including cardiac arrhythmias (Garriott and

²Corresponding Author: Keith L. Shelton, P.O. Box 980613, Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298-0613, Phone: 804/827-2104, Fax: 804/282-2117, kshelto@vcu.edu.

Petty, 1980), B-12 deficiency myelopathy (Hathout and El- Saden, 2011) and cardiorespiratory failure (Potocka-Banas et al., 2011). Acute use risks include skin irritation and necrosis (Hwang et al., 1996).

N₂O is a member of a larger heterogeneous grouping of abused inhalants classified by their shared route of administration. However inhalants can be segregated according to other criteria including product type, chemical class or pharmacological properties (Balster et al., 2009). Grouping inhalants according to their abuse-related pharmacological properties is likely the most relevant classification scheme for the drug abuse research and treatment community but the present literature is not yet sufficient for this purpose (Balster et al., 2009). Our laboratory has for the past several years been employing the drug discrimination to classify inhalants according to their abuse-related subjective stimulus effects. We have previously focused on abused volatile solvents and anesthetics (Shelton, 2007, 2009, 2010; Shelton and Nicholson, 2010, 2012; Shelton and Slavova-Hernandez, 2009). Unlike these inhalants, N₂O does not suppress locomotor activity at higher concentrations, fails to produce sedation and does not alter heart rate (Steffey et al., 1974). Also unlike most other inhalants N₂O is an analgesic and human abusers often describe sensory distortions more characteristic of hallucinogens than CNS depressants (Block et al., 1990). These data suggest that N₂O may not be acting via the same neurochemical mechanisms as other inhalants and thus may represent a unique subclass of abused inhalants.

Only a few studies have examined N₂O in a drug discrimination paradigm. N₂O has been tested in animals trained to discriminate isoflurane, TCE, morphine and ethylketocyclazocine (Hynes and Hymson, 1984; Shelton and Nicholson, 2010, 2012). N₂O partially substituted in mice trained to discriminate the abused chlorinated hydrocarbon vapor TCE from air (Shelton and Nicholson, 2012) but failed to substitute in mice trained to discriminate the volatile anesthetic isoflurane from air (Shelton and Nicholson, 2010). The stimulus effects of N₂O also overlapped with those of the kappa opioid agonist ethylketocyclazocine but not the mu opioid agonist morphine (Hynes and Hymson, 1984).

These results of previous studies demonstrate that N₂O has detectable stimulus effects but no studies have yet been published in which N₂O itself served as a training stimulus. Therefore, the present study aimed to determine if it was possible to establish a N₂O discrimination in mice and, if so, to characterize the temporal properties of its discriminative stimulus effects. Additionally, we wished to examine the degree to which the stimulus effects of nitrous oxide overlap with those of other abused inhalants. These data would help clarify whether nitrous oxide represents a unique subclass of abused inhalants (Balster, 1998) or, instead, is more closely related to other abused inhalants such as volatile solvents and anesthetics.

Methods

Subjects

Sixteen naïve adult male B6SJLF1/J mice (The Jackson Laboratory, Bar Harbor, Maine) served as subjects. This strain has been used extensively in prior inhalant drug discrimination studies conducted in our laboratory (Shelton, 2007, 2009, 2010; Shelton and Nicholson, 2010; Shelton and Slavova-Hernandez, 2009). The mice were individually housed on a 12 h light/dark cycle (lights on 06:00 h). To promote operant responding, the mice were maintained at 85% of their free-feeding weights. Subjects were fed 2.0–3.0 grams/day in their home cage post session and had free access to water except during experimental sessions. Studies were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University and conducted in accordance with the

Institute of Laboratory Animal Research “Guide for the Care and Use of Laboratory Animals” (“Guide For The Care and Use of Laboratory Animals,” 2011).

Apparatus

Operant sessions were conducted in standard two-lever mouse operant conditioning chambers equipped with 0.01-ml liquid dippers (model ENV-307AW; MED Associates, St. Albans, VT). A yellow LED lever was located above each lever. A single 5-Watt LED house light was located at the top center of the chamber rear wall. Drug discrimination schedule conditions and data recordings were controlled by a MED Associates interface and MED-PC version 4 software (MED Associates, St Albans, VT). Mice were reinforced with a solution of 25% sugar, 25% nonfat powdered milk, and 50% tap water by volume.

A 26 liter acrylic exposure chamber encased each operant conditioning chamber. Delivery of N₂O was accomplished by a manually-controlled gas mixing system. An oxygen concentrator supplied O₂ while a medical grade compressed N₂O cylinder and regulator supplied N₂O. Flows of both O₂ and N₂O were controlled by individual calibrated rotometers (Matheson Tri-Gas, Montgomeryville, PA). O₂ and N₂O were combined prior to passing into the inlet fitting of the inhalant chamber and waste gas was expelled through an outlet fitting into a fume hood. Components were connected with flexible Tygon tubing (Fisher Scientific, Hampton, NH). To test volatile vapors each exposure cubicle also contained an 80mm 24v DC fan mounted in an acrylic frame with a perforated metal filter paper attachment grill. Volatile vapor exposures were delivered by injecting a calculated volume of volatile liquid on to filter paper and activating the fan. Injection volume calculations were applications of the ideal gas law using the molar mass and density of the vapor assuming standard ambient temperature and pressure [for more details see (Shelton, 2007)].

Training and substitution test procedure

Mice were trained once daily Mon-Fri to discriminate 60% N₂O+40% O₂ from 100% O₂. The first 10 min period of each session was a timeout in which gas delivery was initiated and the chamber concentration allowed to equilibrate. After the 10 min timeout, the house light and lever lights were illuminated and a 5-min operant session began. During the operant session, completion of the fixed ratio (FR) requirement on the active lever resulted in 3 sec of access to a 0.01 ml milk dipper. Responding on the inactive lever reset the FR requirement on the correct lever. N₂O and vehicle (O₂) training sessions were presented in a double alternation sequence (O₂, O₂, N₂O, N₂O). Over sessions the FR response requirement was increased to FR12. Training continued under these conditions until mice acquired the discrimination. Acquisition was defined as emission of the first FR on the correct lever and greater than 80% of all responses on the correct lever for 8 of 10 consecutive training sessions. After acquisition, substitution tests were conducted on Tue and Fri, provided that the mice continued to exhibit accurate stimulus control on the Mon, Wed, Thur training sessions. If a mouse failed to maintain adequate stimulus control it received additional training sessions until criteria for stability were regained, i.e., the first FR was emitted on the correct lever and greater than 80% of all responses on the correct lever for three consecutive training sessions. On test days, both levers were active and completion of the FR requirement on either lever was reinforced. Concentration/doses were administered in ascending order. When possible doses or concentrations were increased until maximal substitution was apparent or a test condition resulted in a greater than 50% suppression of responding compared to the O₂ control.

To assess the rate of onset of the stimulus effects of N₂O, mice were exposed to 60% N₂O +40% O₂ training concentration for 1, 3, 7 or 10 min prior to the start of the discrimination

test session. The offset of the stimulus effects of N₂O was examined by delaying the start of the discrimination test session for 1, 3 or 5 min after the cessation of 10 min of exposure to 60% N₂O+40%. To approximate if the training exposure duration of 10 min was sufficient to produce steady-state tissue concentrations of N₂O, the training exposure duration of 10 min was also compared to an exposure duration of 20 min. Each substitution concentration-effect or dose-effect curve was preceded by 100% O₂ and 60% N₂O+40% O₂ control sessions. Control test sessions for ethanol and D-amphetamine were preceded by vehicle administration.

Drugs

Medical nitrous oxide was obtained from National Welders Supply (Richmond, VA). Oxygen was supplied by an Airsep Onyx+ oxygen concentrator. Toluene, 1,1,1-trichloroethane (TCE) and 2-butanol were purchased from Sigma-Aldrich Chemicals (St-Louis, MO, USA). Isoflurane was purchased from Webster Veterinary Supply (Charlotte, NC, USA). Methoxyflurane was obtained from Pitman-Moore (Mundelein, IL, USA) and 95% ethanol was obtained from Acros Organics (Fair Lawn, NJ, USA). D-amphetamine was obtained from the NIDA Drug Supply Program (Bethesda, MD, USA). Ethanol and D-amphetamine were prepared in 0.9% saline and injected at a volume of 10 ml/kg. Except as indicated, N₂O exposures were begun 10 min prior to the start of operant session and continued for the duration of the operant test session. Isoflurane, methoxyflurane, toluene or TCE exposures were begun 10 min pre-session and continued for the duration of the 5-min test session. Ethanol and D-amphetamine were injected i.p. 10 min prior to the operant test session.

Data analysis

The percentage correct-lever responding and response rates were calculated for each animal and averaged ($\pm$ SEM) across the group. Results for any drug dose or concentration that suppressed response rates such that a mouse did not complete at least one FR during the test session were excluded from analysis and presentation of the group N₂O discrimination but retained in the analysis and presentation of group response rates. Test concentrations/doses at which fewer than 3 mice emitted a FR during the test session are not shown in the percentage total N₂O-lever data presentation. Full substitution for the 60% N₂O+40% O₂ training condition was defined as eighty percent or greater N₂O-appropriate responding, whereas incomplete substitution was defined as 20% to 79% mean N₂O-lever responding. Mean N₂O-lever responding below 20% was considered no substitution. For compounds that engendered greater than 50% N₂O appropriate responding, EC₅₀ or ED₅₀ values (and 95% confidence limits) were calculated based on the linear portion of each mean dose-effect curve. Response rate data were analyzed using within-subject repeated measures analysis of variance and Dunnett's post hoc tests to assess suppression of operant responding. Effects were considered significant when $p < 0.05$.

Results

All sixteen mice acquired the 60% N₂O+40% O₂ versus 100% O₂ discrimination in a mean of 40 ($\pm$ 3.5) training sessions. Individual subjects required between 24–71 training sessions to meet the training criteria. Figure 1 shows the percentage of subjects meeting both criteria for each training condition from day 1 through day 71 plotted as two-day means. There appeared to be no difference in the rate at which the 100% O₂ and 60% N₂O+40% O₂ conditions were acquired (Figure 1). N₂O concentration-dependently and fully substituted for the 60% training concentration with an EC₅₀ value of 20% (C.L. 14 – 28%) (Figure 2, upper panel). Control tests with 100% O₂ and 60% N₂O occasioned 3% ($\pm$ 1) and 94% ($\pm$ 2) N₂O-appropriate responding, respectively. N₂O had little effect on rates of responding

across the range of concentrations tested (Figure 2, lower panel). As compared to the O₂ control, significant ($p < 0.05$) decreases in operant response rates only occurred following 80% N₂O exposure.

The upper panel of Figure 3 shows the mean ($\pm$ SEM) percentage N₂O-lever responding produced by varying the duration of 60% N₂O exposure. The data indicate that a seven min exposure resulted in 93% N₂O-lever responding, whereas exposure durations of 3 and 1 min reduced N₂O-appropriate responding to 44% (± 8) and 24% (± 6), respectively. The lower panel of figure 3 shows the consequences of 10 min exposure to the 60% N₂O+40% O₂ training condition following increased periods of delay of the 5 min operant test session. Whereas starting the operant session immediately after ceasing exposure (0 minute delay) resulted in 97% (± 2) N₂O-appropriate responding, delaying the start of the session by 1 and 3 min resulted in 84% (± 14) and 59% (± 23) N₂O-appropriate responding, respectively. Delaying the start of the discrimination test session by 5 min reduced N₂O-appropriate responding to near that produced by O₂. In order to study the role of duration of exposure at a given N₂O exposure concentration, substitution tests were conducted with 30% N₂O exposure for 10 and 20 min as well as the training condition of 60% N₂O exposure for 10 min (Table 1). Extended exposure to 30% N₂O for 20 min did not appreciably increase mean percent N₂O-lever responding over that produced by 10 min of 30% N₂O exposure suggesting maximal expression of behavioral effects was achieved with 10 min of exposure.

The chlorinated hydrocarbon vapor 1,1,1-trichloroethane (TCE) incompletely substituted for 60% N₂O up to a maximum of 44% (± 18) at 12000 ppm (Figure 4, upper panel closed circles). TCE attenuated response rates in a concentration-dependent manner ($F_{(7,28)} = 6.243$, $p < 0.05$) (Figure 4, lower panel) with an EC₅₀ of 8264 ppm (C.L. 7295 – 9361 ppm). The aromatic hydrocarbon vapor toluene produced a maximum 72% (± 10) N₂O-lever selection at 8000 ppm (Figure 4, upper panel closed squares) and, like TCE, also attenuated operant response rates in a concentration-dependent manner ($F_{(7,35)} = 8.853$, $p < 0.05$) (Figure 4, lower panel). The EC₅₀ value of substitution and response rate suppression by toluene were 3174 ppm (C.L. 1970 – 5117 ppm) and 5192 ppm (C.L. 4,509 – 5,977 ppm), respectively.

The volatile halogenated anesthetic isoflurane (Figure 5, upper panel, closed circles) substituted for N₂O. to a lesser degree than TCE or toluene. Isoflurane produced a concentration-dependently increase in the percentage N₂O-lever responding to a maximum of only 39% (± 11) at 4000 ppm. Higher concentrations were not studied because isoflurane also produced concentration-dependent decreases in operant responding ($F_{(7,28)} = 4.814$, $p < 0.05$) (Figure 5, lower panel), with an EC₅₀ of 3803 ppm (C.L. 3218 – 4496 ppm). The volatile halogenated anesthetic methoxyflurane (Figure 5, upper panel, closed squares) similarly produced a maximum of 47% (± 14.2) N₂O-lever responding at the concentration of 2000 ppm and, also, decreased operant responding concentration-dependent manner ($F_{(8,32)} = 1.685$, $p < 0.05$) (Figure 5, lower panel), with an EC₅₀ of 1902 ppm (C.L. 1563 – 2316 ppm). Ethanol (Figure 5, upper panel, closed triangles) dose dependently increased N₂O-lever responding to a maximum of 52% (± 14) at 2.5 g/kg. Ethanol also dose-dependently decreased response rates ($F_{(7,28)} = 3.182$, $p < 0.05$) with an ED₅₀ of 1.2 g/kg (C.L. 0.62 - 2.44 g/kg). Finally, D-amphetamine up to a dose of 1.56 mg/kg produced no greater than 1% N₂O-lever responding (data not shown). The odorant, 2-butanol up to a concentration of 100 ppm, which is 100 times greater than that detectible in mice (Laska and Shepherd, 2007), failed to produce greater than 3% N₂O-lever selection.

Discussion

Previous studies have demonstrated that abused volatile vapor inhalants can serve as discriminative stimuli either when administered by injection or inhalation (Knisely et al., 1990; Shelton, 2007, 2009, 2010; Shelton and Nicholson, 2010; Shelton and Slavova-Hernandez, 2009). However, this is the first study of which we are aware in which a gas, specifically N_2O , has been trained as a discriminative stimulus in nonhuman subjects. Although nitrous oxide is generally inhaled alone when abused, extended exposure to nitrous oxide in a clinical setting is always supplemented with oxygen to prevent hypoxia. Since our training exposure duration was 10 min, we also administered our 60% N_2O training condition combined with 40% oxygen. For technical reasons and simplicity, we used 100% O_2 as the alternative training condition. If O_2 itself had stimulus effects, the interpretation of cross-substitution results in the present discrimination studies might be unduly complicated (Overton, 1982; Overton et al., 1989). However, 100% O_2 , when compared with compressed air, does not appear to affect mood or psychomotor performance. Additionally, other studies have used O_2 as a vehicle in the discrimination of 100% O_2 from N_2O concentrations (Dohrn et al., 1992, 1993; Zacny et al., 1994; Walker and Zacny, 2003; Kangas and Walker, 2008). Therefore, it is likely that in the present training conditions, the stimulus effect of O_2 are negligible and that the present discrimination is functionally an N_2O versus vehicle discrimination. Finally, for technical reasons related to calculating appropriate vapor test concentrations, air was used as the control condition in tests with volatile compounds. In all cases these air control tests were generalized to the O_2 lever.

N_2O fully substituted for 10 min exposure to 60% N_2O in a concentration-dependent manner (figure 2, upper panel). With the maximum test concentration of approximately 80%, we were unable to demonstrate more than modest response rate suppression by N_2O (figure 2, lower panel). This is unsurprising in view of its minimum alveolar concentration (MAC) of 105% for anesthesia in humans (Steffey et al., 1974). Previous N_2O versus placebo choice paradigms in humans indirectly estimated a 5 min onset of stimulus effects (Walker and Zacny, 2003; Kangas and Walker, 2008; Zacny et al., 2008) and subjective effects ratings on the pentobarbitalchlorpromazine- alcohol group (PCAG) and lysergic acid diethylamide (LSD) Addiction Research Center inventory (ARCI) scales were significantly increased 15 and 29 min after the initiation of N_2O inhalation (Dohrn et al., 1993). In mice, 7 min of N_2O exposure was necessary to engender full substitution for the 10 min training exposure in the present study, which is consistent with human data. However, the offset of N_2O effects in mice appears to be more rapid in mice than humans. In human subjects, drug liking was still elevated 40 min after a 1 hour exposure to 30% or 40% N_2O (Zacny et al., 1996) whereas the stimulus effects of N_2O in mice were near vehicle levels only 5 min after the cessation of gas exposure. The more rapid offset but similar onset of N_2O effects in mice and humans is curious. One potential factor may be exposure duration: that in the previous human studies, the exposure duration was 1 h but in our studies the training exposure duration was only 10 min. It could be the case that 10 min is insufficient to fully saturate the tissue of the mice, resulting in a more rapid offset. On the other hand, our data showing that there was no difference in the degree of substitution elicited by 30% nitrous oxide as a result of doubling the exposure duration suggests that 10 min of N_2O exposure in mice is sufficient to reach steady state levels. Other factors such as relative body mass and respiration rate may be more salient to these noted differences across species.

The rapid offset of N_2O also may explain why we previously were unable to establish a N_2O versus vehicle discrimination using methods that had been successful in training volatile solvents and vapor anesthetics as discriminative stimuli. In prior inhalant discrimination studies we exposed mice to their respective training inhalants for 10 min and then removed

them from the exposure chamber and conducted training and testing in standard operant chambers (Shelton, 2007, 2009). When we used the same procedure with N₂O it required in excess of 100 training sessions to establish the discrimination and the reliability of the subjects on training days was extremely low. In contrast, when subjects were trained while concurrently exposed to N₂O, the number of training sessions required to meet acquisition criteria was comparable to that of other inhalants (Shelton, 2007, 2009; Shelton and Nicholson, 2010). These observations indicate that, independent of its kinetics, the stimulus effects of 60% nitrous oxide in mice are highly salient.

A categorization system of inhalants based on intended usage and general chemical characteristics has been proposed (Balster, 1998; Balster et al., 2009). This system hypothesizes that inhalants fall into three categories: volatile alkyl nitrites, volatile solvents/fuels/anesthetics and N₂O. One goal of the present study was to determine using drug discrimination if the *in vivo* abuse-related pharmacological properties of N₂O justify it being placed in a separate category from these other compounds (Balster, 1998). Based upon results with the abused volatile solvents toluene and TCE in the present experiments, the discriminative-stimulus effects of toluene and N₂O overlap considerably with those of toluene and to a lesser extent with those of TCE, suggesting they might be considered in the same category. On the other hand, the discriminative-stimulus effects of N₂O overlapped only marginally with those of the volatile anesthetics isoflurane and methoxyflurane. This separation is consistent with previous studies in which, using 'subjective effects' questionnaires, normal human volunteers describe isoflurane as sedating (Zacny et al., 1994) whereas N₂O produces more "high" "tingling" and "dreamy, detached reverie" (Zacny et al., 1994; Beckman et al., 2006). Unlike isoflurane, both methoxyflurane and N₂O have pronounced analgesic effects in humans (Tomi et al., 1993; Abdullah et al., 2011; Caldicott, 2011). The limited degree of substitution produced by methoxyflurane suggests that shared analgesic properties do not appreciably contribute to N₂O-like stimulus effects.

The results with ethanol were of interest because ethanol previously has been shown to have some neurochemical and behavioral effects in common with N₂O, abused solvents, and volatile anesthetics (Lapin, 1993; Bowen et al., 1996a, 1996b; Boehm et al., 2002). In the present studies, however, ethanol elicited only marginally greater N₂O-lever selection than did the two volatile anesthetic agents. These data suggest that any commonality in the behavioral effects of ethanol and N₂O may extend only minimally to their subjective effects. Finally, given that toluene produced a high degree of N₂O-like effects and that previous studies have shown that toluene elicits partial substitution in D-amphetamine trained mice (Bowen, 2006), we were interested in whether D-amphetamine might produce N₂O-like stimulus effects. However, in the present study D-amphetamine produced only vehicle-appropriate responding, demonstrating that the stimulus effects of N₂O are pharmacological specific.

Studies have suggested that the analgesic, anxiolytic and anesthetic effects of nitrous oxide may be mediated by opioid, GABA_A and NMDA receptors, respectively (for review see Emmanouil and Quock, 2007). It is unclear if these same neurotransmitter systems play a role in the abuse-related discriminative stimulus effects of nitrous oxide. Both NMDA and GABA_A receptor involvement are possibilities, given the present data showing substantial overlap in the stimulus effects of nitrous oxide and toluene, which has been shown to affect the same three neurotransmitter systems (reviewed by Cruz et al. 2011). We have previously shown that the discriminative stimulus effects of both isoflurane and TCE have a significant GABA_A positive modulator-like component (Shelton and Nicholson, 2010, 2012). Thus, given the overlap in stimulus effects, the involvement of GABA_A receptors in the effects of nitrous oxide is certainly possible. A primary role of GABA_A receptors in the stimulus effects of nitrous oxide, however, appears unlikely, given that both isoflurane and TCE only

weakly substituted for N₂O in the present study and N₂O failed to produce either isoflurane or TCE-like stimulus effects in mice trained to discriminate those inhalants from air (Shelton and Nicholson, 2010, 2012). The differing cross-substitution pattern between nitrous oxide, TCE and isoflurane in the present study and prior experiments is curious. Similar asymmetrical cross-substitution previously has been reported between ethanol, GABA_A positive modulators and NMDA antagonists (Schechter and Lovano, 1985; Grant and Colombo, 1993). In this regard, the stimulus effects of ethanol are composed of both GABA_A positive modulatory effects as well as NMDA antagonist effects. In ethanol-trained subjects, both GABA_A positive modulators as well as NMDA antagonists produce full substitution (Järbe and McMillan, 1983; Schechter and Lovano, 1985; Grant and Colombo, 1993; Shelton and Balster, 1994; Shelton and Grant, 2002), whereas ethanol substitutes poorly in animals trained to discriminate either GABA_A positive modulators or NMDA antagonists from vehicle (York, 1978; Järbe and McMillan, 1983). Such results have been ascribed to the nature of mixture discriminations in which the components of a mixture are discriminated separately rather than as a new and unique stimulus complex (Stolerman, et al., 1987). Thus, any salient component of a discriminative stimulus produced by a mixture should elicit substitution. However, when a mixture is tested in animals trained to discriminate only one of its components, the other components in the mixture may overshadow the stimulus effects of the training component (Mariathasan et al., 1991; Harrison et al., 1998). With this framework in mind, we speculate that the discriminative stimulus of nitrous oxide may result from multiple neurochemical actions, with a prominent action shared with toluene but only a minor one shared with isoflurane and TCE. Additional studies specifically exploring the receptor systems underlying the stimulus effects of nitrous oxide will be necessary to determine which receptor mechanisms contribute to its stimulus effects as well as to further consider whether N₂O should be categorized as fundamentally different from other abused inhalants.

References

- Abdullah W, Sheta S, Nooh N. Inhaled methoxyflurane (Penthrox) sedation for third molar extraction: a comparison to nitrous oxide sedation. *Aust Dent J.* 2011; 56(3):296–301. [PubMed: 21884146]
- Balster R, Cruz S, Howard M, Dell C, Cottler L. Classification of abused inhalants. *Addiction.* 2009; 104(6):878–882. [PubMed: 19215599]
- Balster R. Neural basis of inhalant abuse. *Drug Alcohol Depend.* 1998; 51(1–2):207–214. [PubMed: 9716942]
- Beckman NJ, Zacny JP, Walker DJ. Within-subject comparison of the subjective and psychomotor effects of a gaseous anesthetic and two volatile anesthetics in healthy volunteers. *Drug Alcohol Depend.* 2006; 81(1):89–95. [PubMed: 16024183]
- Bell D, McDonough J, Ellison J, Fitzhugh E. Controlled drug misuse by Certified Registered Nurse Anesthetists. *AANA J.* 1999; 67(2):133–140. [PubMed: 10488287]
- Block R, Ghoneim M, Kumar V, Pathak D. Psychedelic effects of a subanesthetic concentration of nitrous oxide. *Anesthesia Progress.* 1990; 37(6):271. [PubMed: 2097905]
- Boehm SL, Reed CL, McKinnon CS, Phillips TJ. Shared genes influence sensitivity to the effects of ethanol on locomotor and anxiety-like behaviors, and the stress axis. *Psychopharmacology.* 2002; 161(1):54–63. [PubMed: 11967631]
- Bowen S. Increases in amphetamine-like discriminative stimulus effects of the abused inhalant toluene in mice. *Psychopharmacology.* 2006; 186(4):517–524. [PubMed: 16601996]
- Bowen SE, Wiley JL, Balster RL. The effects of abused inhalants on mouse behavior in an elevated plus-maze. *Eur J Pharmacol.* 1996a; 312(2):131–136. [PubMed: 8894586]
- Bowen SE, Wiley JL, Evans EB, Tokarz ME, Balster RL. Functional observational battery comparing effects of ethanol, 1, 1, 1-trichloroethane, ether, and flurothyl. *Neurotoxicol Teratol.* 1996b; 18(5): 577–585. [PubMed: 8888022]
- Caldicott DGE. Methoxy—what?! *Emerg Med J.* 2011; 28(8):726–726. [PubMed: 21406575]

- Cruz SL. The latest evidence in the neuroscience of solvent misuse: an article written for service providers. *Subst Use Misuse*. 2011; 46(Suppl 1):62–7. [PubMed: 21609148]
- Dohrn C, Lichtor J, Finn R, Uitvlugt A, Coalson D, Rupani G, de Wit H, et al. Subjective and psychomotor effects of nitrous oxide in healthy volunteers. *Behav Pharmacol*. 1992; 3(1):19–30. [PubMed: 11224098]
- Dohrn C, Lichtor J, Coalson D, Uitvlugt A, de Wit H, Zacny J. Reinforcing effects of extended inhalation of nitrous oxide in humans. *Drug Alcohol Depend*. 1993; 31(3):265–280. [PubMed: 8462415]
- Emmanouil DE, Quock RM. Advances in understanding the actions of nitrous oxide. *Anesth Prog*. 2007; 54(1):9–18. [PubMed: 17352529]
- Garriott J, Petty C. Death from inhalant abuse: toxicological and pathological evaluation of 34 cases. *Clin Toxicol*. 1980; 16(3):305–315. [PubMed: 7398219]
- Grant K, Colombo G. Discriminative stimulus effects of ethanol: effect of training dose on the substitution of N-methyl-D-aspartate antagonists. *J Pharmacol Exp Ther*. 1993; 264(3):1241–1247. [PubMed: 8450461]
- Guide For The Care and Use of Laboratory Animals. National Academies Press; 2011.
- Harrison Y, Jenkins J, Rocha B, Lytle D, Jung M, Oglesby M. Discriminative stimulus effects of diazepam, ketamine and their mixture: ethanol substitution patterns. *Behav Pharmacol*. 1998; 9(1):31–40. [PubMed: 9832946]
- Hathout L, El-Saden S. Nitrous oxide-induced B12 deficiency myelopathy: Perspectives on the clinical biochemistry of vitamin B12. *J Neurol Sci*. 2011; 301(1–2):1–8. [PubMed: 21112598]
- Hwang J, Himel H, Edlich R. Frostbite of the face after recreational misuse of nitrous oxide. *Burns*. 1996; 22(2):152–153. [PubMed: 8634126]
- Hynes MD, Hymson DL. Nitrous oxide generalizes to a discriminative stimulus produced by ethylketocyclazocine but not morphine. *Eur J Pharmacol*. 1984; 105(1):155–159. [PubMed: 6149142]
- Järbe T, McMillan D. Interaction of the discriminative stimulus properties of diazepam and ethanol in pigeons. *Pharmacol Biochem Behav*. 1983; 18(1):73–80. [PubMed: 6828540]
- Kangas BD, Walker DJ. An adjusting-dose procedure for assessing the reinforcing effects of nitrous oxide with humans. *Pharmacol Biochem Behav*. 2008; 91(1):104–108. [PubMed: 18639578]
- Knisely J, Rees D, Balster R. Discriminative stimulus properties of toluene in the rat. *Neurotoxicol Teratol*. 1990; 12(2):129–133. [PubMed: 2333064]
- Lapin I. Anxiogenic effect of phenylethylamine and amphetamine in the elevated plusmaze in mice and its attenuation by ethanol. *Pharmacol Biochem Behav*. 1993; 44(1):241–243. [PubMed: 8094251]
- Laska M, Shepherd GM. Olfactory discrimination ability of CD-1 mice for a large array of enantiomers. *Neuroscience*. 2007; 144(1):295–301. [PubMed: 17045753]
- Mariathasan EA, Garcha HS, Stolerman IP. Discriminative stimulus effects of amphetamine and pentobarbitone separately and as mixtures in rats. *Behav Pharmacol*. 1991; 2(4–5):405–415. [PubMed: 11224083]
- Overton D. Multiple drug training as a method for increasing the specificity of the drug discrimination procedure. *J Pharmacol Exp Ther*. 1982; 221(1):166–172. [PubMed: 7062280]
- Overton D, Shen C, Ke G, Gazdick L. Discriminable effects of phencyclidine analogs evaluated by multiple drug (PCP versus OTHER) discrimination training. *Psychopharmacology*. 1989; 97(4):514–520. [PubMed: 2498947]
- Potocka-Banas B, Majdanik S, Dutkiewicz G, Borowiak K, Janus T. Death caused by addictive inhalation of nitrous oxide. *Hum Exp Toxicol*. 2011; 30(11):1875–1877. [PubMed: 21393349]
- Schechter MD, Lovano DM. Ethanol-chlordiazepoxide interactions in the rat. *Pharmacol Biochem Behav*. 1985; 23(6):927–930. [PubMed: 4080778]
- Seidberg BH, Sullivan T. Dentists' use, misuse, abuse or dependence on mood-altering substances. *N Y State Dent J*. 2004; 70(4):30–33. [PubMed: 15270312]
- Shelton K. Inhaled toluene vapor as a discriminative stimulus. *Behav Pharmacol*. 2007; 18(3):219–229. [PubMed: 17426486]

- Shelton K. Discriminative stimulus effects of inhaled 1,1,1-trichloroethane in mice: comparison to other hydrocarbon vapors and volatile anesthetics. *Psychopharmacology*. 2009; 203(2):431–440. [PubMed: 18972104]
- Shelton K. Pharmacological characterization of the discriminative stimulus of inhaled 1,1,1-trichloroethane. *J Pharmacol Exp Ther*. 2010; 333(2):612–620. [PubMed: 20123932]
- Shelton K, Balster R. Ethanol drug discrimination in rats: substitution with GABA agonists and NMDA antagonists. *Behav Pharmacol*. 1994; 5(4 And 5):441–451. [PubMed: 11224296]
- Shelton K, Grant K. Discriminative stimulus effects of ethanol in C57BL/6J and DBA/2J inbred mice. *Alcohol Clin Exp Res*. 2002; 26(6):747–757. [PubMed: 12068241]
- Shelton K, Nicholson K. GABA A positive modulator and NMDA antagonist-like discriminative stimulus effects of isoflurane vapor in mice. *Psychopharmacology*. 2010; 212(4):559–569. [PubMed: 20697696]
- Shelton K, Nicholson K. GABA_A-positive modulator selective discriminative stimulus effects of 1,1,1-trichloroethane vapor. *Drug Alcohol Depend*. 2012; 121:103–109. [PubMed: 21924562]
- Shelton K, Slavova-Hernandez G. Characterization of an inhaled toluene drug discrimination in mice: effect of exposure conditions and route of administration. *Pharmacol Biochem Behav*. 2009; 92(4): 614–620. [PubMed: 19268500]
- Steffey E, Gillespie J, Berry J, Eger E, Munson E. Anesthetic potency (MAC) of nitrous oxide in the dog, cat, and stump-tail monkey. *J Appl Physiol*. 1974; 36(5):530–532. [PubMed: 4826313]
- Stolerman IP, Rauch RJ, Norris EA. Discriminative stimulus effects of a nicotine-midazolam mixture in rats. *Psychopharmacology*. 1987; 93(2):250–6. [PubMed: 3122258]
- Substance Abuse and Mental Health Services Administration, Office of Applied Studies. The NSDUH Report: Inhalant Use across the Adolescent Years. Rockville, MD: 2008.
- Tomi K, Mashimo T, Tashiro C, Yagi M, Pak M, Nishimura S, Nishimura M, et al. Alterations in pain threshold and psychomotor response associated with subanaesthetic concentrations of inhalation anaesthetics in humans. *Br J Anesth*. 1993; 70(6):684– 686.
- Walker DJ, Zacny JP. Bitonic dose-response functions for reinforcing and self-reported effects of nitrous oxide in humans. *Pharmacol Biochem Behav*. 2003; 74(4):851–857. [PubMed: 12667899]
- York JL. A comparison of the discriminative stimulus effects of ethanol, barbital, and phenobarbital in rats. *Psychopharmacology*. 1978; 60(1):19–23. [PubMed: 104341]
- Zacny J, Sparacino G, Hoffmann P, Martin R, Lichtor J. The subjective, behavioral and cognitive effects of subanesthetic concentrations of isoflurane and nitrous oxide in healthy volunteers. *Psychopharmacology*. 1994; 114(3):409–416. [PubMed: 7855199]
- Zacny J, Lichtor J, Coalson D, Alessi R, Goldsher G, Young C, Apfelbaum J, et al. Examining the subjective, psychomotor and reinforcing effects of nitrous oxide in healthy volunteers: a dose-response analysis. *Behav Pharmacol*. 1996; 7(2):194–199. [PubMed: 11224411]
- Zacny J, Walker D, Derus L. Choice of nitrous oxide and its subjective effects in light and moderate drinkers. *Drug Alcohol Depend*. 2008; 98(1–2):163–168. [PubMed: 18640794]

Fig 1.

Percentage of mice meeting both the correct first fixed ratio and greater than 80% total training-appropriate responding across consecutive training sessions (n=16). Each point represents two-day means for O₂ (open circles) and N₂O (closed circles).

Fig 2. Concentration-effect curve of N₂O in mice trained to discriminate 60% N₂O+40% O₂ from 100% O₂ (n=16). Points above O₂ and N₂O represent 100% O₂ (open circle) and 60% N₂O +40% O₂ (closed circle) control test sessions. Upper panel: mean (±SEM) percentage N₂O-lever responding; lower panel: mean (±SEM) response rates.

Fig 3.

Upper panel: mean ($\pm$ SEM) percentage N₂O-lever responding produced by increasing durations of exposure to 60% N₂O+40% O₂ (n=9). Lower panel: mean ($\pm$ SEM) percentage N₂O-lever responding following increasing durations of delay after cessation of 10 min of exposure to 60% N₂O+40% O₂ (lower panel, n=5). Points above O₂ are the results of 100% O₂ control sessions.

Fig 4.

Concentration-effect curves of TCE (n=8) and toluene (n=8) vapor in mice trained to discriminate 10 min exposure to 60% N₂O+40% O₂ from 100% O₂. Points above O₂ represent control sessions. Points above N₂O represent 60% N₂O control test sessions. Upper panel: mean ($\pm$ SEM) percentage N₂O-lever responding for TCE (closed circles) and toluene (closed squares). Lower panel: mean ($\pm$ SEM) response rates (responses/s). Numbers in parentheses indicate the number of subjects within the group which emitted at least one complete FR at that test concentration. * indicate statistically significant ($p < 0.05$) differences from O₂ control session.

Fig 5.

Concentration-effect curves of isoflurane (n=8) and methoxyflurane (n=9) vapor and dose-effect curve of ethanol (n=8) in mice trained to discriminate 10 min exposure to 60% N₂O+40% O₂ from 100% O₂. Points above O₂ represent O₂ control sessions. Points above N₂O represent 60% N₂O+40% O₂ control test sessions. Upper panel: mean ($\pm$ SEM) percentage N₂O-lever responding for isoflurane (closed circles), methoxyflurane (closed squares) and ethanol (closed triangles). Lower panel: mean ($\pm$ SEM) response rates (responses/s). Numbers in parentheses indicate the number of subjects within the group which emitted at least one complete FR at that test concentration. * indicate statistically significant ($p < 0.05$) differences from O₂ control session.

Table 1

Mean ($\pm$ SEM) percent N₂O-lever selection following exposure to the training concentrations of 100% O₂ or 60% N₂O for 10 min as well as exposure to 10 or 20 min of exposure to 30% N₂O.

Exposure condition and duration	% N ₂ O-lever responding
	Mean ($\pm$ SEM)
100% O ₂ – 10 min	1 (0.6)
60% N ₂ O – 10 min	91 (2.5)
30% N ₂ O – 10 min	41 (9.4)
30% N ₂ O – 20 min	37 (10.3)