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Conditioned place aversion and self-administration of nitrous oxide in rats

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Abstract

The rewarding/aversive effects of nitrous oxide (N_2O) were evaluated using the place conditioning paradigm. Male Long–Evans rats ($N=103$) received a daily 40-min gas exposure for 8 consecutive days that alternated between two distinct chambers. A control group received placebo gas in both chamber types, while the N_2O groups (8%, 15%, 30%, and 60% N_2O) received four consistent pairings of N_2O with one chamber type and four pairings of placebo gas with the other. A conditioned place aversion was found for the chambers that had been paired with 30% and 60% N_2O. Place aversions were demonstrated during a 20-min test session on Day 9 when placebo gas was delivered to both chambers, and also during a 20-min test session on Day 10 when N_2O was delivered to both chambers. A second study evaluated two novel methods of inhalant self-administration, one that used a forced-choice alternating gas environment and one that used a free-choice paradigm. Of four rats tested, two self-administered N_2O, one rat avoided N_2O, and one rat's behavior was consistent with neither self-administration nor avoidance. Availability of these methods will facilitate research on the neurobiological mechanisms underlying the rewarding and reinforcing effects of N_2O and other abused inhalants.

Keywords: Conditioned place preference (CPP); Place conditioning; Drug reward; Drug reinforcement; Drug abuse; Inhalant

1. Introduction

Nitrous oxide (N_2O) has a long history as an abused drug (Gillman, 1992; Layzer, 1985; Rosenberg et al., 1979). Although N_2O abuse has never become a major social problem when compared with other addictive drugs (Layzer, 1985), it can have serious consequences (Stacy et al., 1992). As an extreme example, people can die from abusing N_2O (Suruda and McGlothlin, 1990; Wagner et al., 1992; Winek et al., 1995). However, when administered under properly controlled conditions, N_2O has an excellent safety record (Gillman, 1982). Consequently, research on N_2O can be conducted using both human and animal subjects.

Like other drugs of abuse (Jasinski et al., 1984), N_2O supports self-administration in animals (i.e., in monkeys, see Grubman and Woods, 1982; Nemeth and Woods, 1982; Wood et al., 1977), and human experiments indicate that N_2O has positively reinforcing effects, although large individual differences exist (Walker and Zacny, 2001, 2002). The procedure used to study N_2O self-administration in squirrel monkeys required the animal to be seated with a gas delivery helmet secured over its head (Wood et al., 1977). After learning to lever press for a 1-min administration of 60% N_2O, subjects administered as many as 200 15-s administrations of N_2O during a 1-h session. High rates of responding could be achieved by manipulating the reinforcement schedule required to receive the N_2O (i.e., 10 or 20 lever presses per gas delivery). Interestingly, monkeys trained to self-administer N_2O also readily self-administered 15-s deliveries of 0.1–0.3% toluene vapor (Weiss et al., 1979). Another published inhalant self-administration technique also used monkeys and employed implanted nasal

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catheters to deliver inhalants (chloroform, ether, and lacquer thinner) when a lever was pressed (Yanagita et al., 1970). These inhalant self-administration methods have not been adapted for use with rats or mice.

Balster's (1998) review article on the neural basis of inhalant abuse states that much of the scientific literature on inhalants has focused on target organ toxicity rather than on the brain and behavior. This lopsided research focus is unfortunate because behavioral pharmacology research is critical to understand the mechanisms underlying inhalant abuse. In comparison with other drugs of abuse, inhalants have been understudied with regards to the neural mechanisms responsible for important addiction-related phenomena such as sensitization, tolerance, dependence, reward, and reinforcement. Research on the neural basis of inhalant reinforcement has been hindered by the lack of an inhalant self-administration method appropriate for rats; indeed, "inhalation self-administration experiments with animals present complex technical problems" (Balster, 1987, p. 9). Although appropriate methods are available for monkeys (Weiss et al., 1979; Wood, 1979; Wood et al., 1977), the typical experimental design requirements make the cost of conducting these experiments with nonhuman primates prohibitively expensive (Campbell and Caroll, 2000). As a consequence, most studies involving drug self-administration are conducted with rats and use non-inhalant drugs by necessity. Thus, there is an obvious need to develop an inhalant self-administration procedure for rats, because it is the "animal procedure which has the most face validity for prediction of abuse potential" (Balster, 1987; p. 8). Another commonly used and less technically complex method to investigate a drug's rewarding/aversive characteristics in the rat is the place conditioning paradigm. However, only a single study (Yavich et al., 1994) has used this approach with an inhalant (i.e., a solvent mixture). The goal of our research was to develop methods suitable for use with rats that would allow place conditioning and self-administration paradigms to be tested with the pharmacologically active gas, N_2O.

2. Experiment 1: place conditioning with N_2O in the rat

There are numerous review articles on place conditioning with drug effects (Bardo and Bevins, 2000; Carr et al., 1989; Swerdlow et al., 1989). It is generally believed that a classically conditioned preference or aversion is acquired for an environmental context based on an animal's history of pairings between the context and drug effect. Presumably, if a drug has rewarding effects, then the animal learns a preference for the drug-associated environment, and if a drug effect is aversive, the animal learns to avoid that environment. After sufficient pairings between the drug and the context, the animal's preference for the drug-paired environment is typically assessed in the absence of the drug, although preference testing can also be conducted in the drugged state to check for a state-dependent memory effect. Another useful characteristic of the place conditioning paradigm is that it can be adapted for use with different species.

Self-administration studies define a drug as reinforcing when an experimental contingency that links a behavior (e.g., lever press, nose-poke) to drug delivery causes the frequency of that behavior to increase. Bardo and Bevins (2000) recommend that place conditioning be described in terms of drug reward rather than drug reinforcement, because it is unclear what behavior is being reinforced during place conditioning. Another important distinction between the place conditioning and self-administration paradigms is that the experimenter administers the drug to the subject during place conditioning while drug delivery is under the control of the animal in self-administration studies. Although place conditioning has sometimes been described as an alternative to drug self-administration, it is clear that these approaches do not provide interchangeable measures of drug reward (Bardo and Bevins, 2000; Deroche et al., 1999). Nevertheless, both procedures are valuable in that they contribute to our understanding of drug reward mechanisms. The purpose of Experiment 1 was to investigate place conditioning in the rat using N_2O.

3. Material and methods for Experiment 1

3.1. Subjects

One hundred and three adult male Long–Evans rats (Simonsen Laboratory, Gilroy, CA) weighing approximately 200–250 g at the start of the experiment were studied. All rats were group housed in a temperature-controlled ($\sim$23 °C) colony room with a 12:12-h light–dark cycle (light cycle from 7:00 a.m. to 7:00 p.m.). Rat chow and water were available ad libitum. The Institutional Review Committee for the use of Animal Subjects approved the procedures used in this study, and the research was conducted in an AAALAC-approved facility.

3.2. Apparatus

Two distinct conditioned place preference (CPP) testing chambers were made from clear polycarbonate tubes (40.6 cm length, 12.1 cm inner diameter): one type of gas administration chamber was clear and smooth, and the other type had a sandpaper floor with vertical black stripes. The clear smooth polycarbonate tube was used without any modifications. The other distinct chamber was created by placing black vertical stripes (electrical tape, 1.3 cm wide, 2.5 cm between each stripe) on the outside surface of a tube. In addition, a tan-colored sandpaper strip (42.0×11.3 cm) with a coarseness of P100 was fixed to the floor of the striped chamber using double-sided adhesive tape. Collars were attached at both

ends of a tube and machined to assure a good fit between the tube and the end caps. The collars made the effective length of the chamber 42.0 cm (approximate volume = 4830 cm^3). Polyethylene end caps were fabricated that fit over the collars on the open ends of each chamber and a bar clamp tightened the end caps on each tube. Rubber O-rings (size #2-250) created a gas-tight seal between each end cap and collar. Gas entered through the top center of each chamber at a flow rate of 2 l/min and was vented via two holes on the top of each chamber located 7.6 cm from either end. All of the lines leaving each chamber and going to the exhaust vent were the same length. Four chambers (two of each type) were constructed and could be used simultaneously.

A gas administration device was constructed to deliver either N_2O or placebo gas independently to each of four chambers. This was accomplished by using three independent gas flow tubes (oxygen, nitrogen, and N_2O) for each chamber. By adjusting the gas flow rate through each of the three flow tubes, it was possible to deliver a precise but different mixture of the three gases to each chamber. The oxygen concentration was 30% of all gas mixtures. The desired N_2O concentration was selected, and the remainder of the gas mixture consisted of nitrogen. Placebo gas consisted of 30% oxygen and 70% nitrogen. The concentration of oxygen and N_2O within the gas mixture delivered to each chamber was verified by use of an infrared gas analyzer (Datex model #CD202; Helsinki, Finland). All gases were medical grade.

3.3. Procedure

Rats (total $N = 103$) received a daily 40-min gas exposure for 8 consecutive days which alternated between the two distinct chamber types. A control group ($n = 24$) received placebo gas in both chambers while the N_2O groups [8% ($n = 19$), 15% ($n = 20$), 30% ($n = 20$), 60% ($n = 20$)] received four consistent pairings of N_2O with one chamber type and four pairings of placebo gas with the other. Half of the rats in each N_2O group were assigned randomly to have N_2O paired with the clear chamber, while the remaining rats had N_2O paired with the striped chamber over the conditioning trials. During a 20-min placebo test session on Day 9, each rat was free to move between the two chamber types that were now connected via a polyethylene open doorway, and the time a rat spent in each side was measured. Placebo gas was delivered to both chambers during this test session. A custom-made optical infrared beam-break unit was used to determine how much time the rat spent in each chamber type. During a 20-min N_2O test session on Day 10, the two distinct chambers were again connected by the open doorway, and the time a rat spent in each side was measured. The concentration of N_2O delivered to both chambers on Day 10 was identical to the concentration administered during N_2O conditioning trials for rats in the N_2O groups, while rats in the control condition were assigned randomly to receive one of the four possible N_2O concentrations for the first time. All experimental procedures took place between 9:00 a.m. and 5:00 p.m.

3.4. Data analysis

During both test sessions, a CPP/aversion for each of the N_2O concentrations was determined by using two-sided t tests to compare the time spent in the clear chamber by rats who had N_2O paired with the clear chamber (N_2O-clear) versus the striped chamber (N_2O-striped). Because the control group received placebo gas in both chambers during the conditioning trials, it was possible to determine whether rats had a preference for one of the chamber types during the initial test session. The effect of N_2O on activity was evaluated using the control rats by comparing the mean number of crossings in the second test session when they received N_2O for the first time with the corresponding mean from the first test session with a paired t test.

4. Results

Rats in the control group exhibited a slight preference for the striped environment during the first test session (mean percent time in the clear chamber was 36.8, 95% confidence interval = 26.7–46.9 which excludes 50). The results of the place conditioning study (Fig. 1) indicate that a conditioned place aversion developed to both 30% and 60% N_2O. During the first test session, rats conditioned with N_2O in the clear chamber (N_2O-clear) spent less time in the clear chamber than did rats conditioned with N_2O in the striped chamber (N_2O-striped) [mean percent time in the clear chamber was 17.5 (N_2O-clear) versus 58.2 (N_2O-striped) for 30% N_2O, $P < .001$; 27.6 (N_2O-clear) versus 74.8 (N_2O-striped) for 60% N_2O, $P = .002$]. Other results showed a slight nonsignificant aversion to 15% N_2O [mean percent time in the clear chamber type was 27.5 (N_2O-clear) versus 43.0 (N_2O-striped), $P = .18$] and a nonsignificant preference for 8% N_2O [mean percent time in the clear chamber type was 46.8 (N_2O-clear) versus 36.6 (N_2O-striped), $P = .38$]. Results from the second test session were similar [mean percent time in the clear chamber type was: 12.4 (N_2O-clear) versus 67.5 (N_2O-striped) for 30% N_2O, $P < .001$; 26.2 (N_2O-clear) versus 61.1 (N_2O-striped) for 60% N_2O, $P = .046$], except that the preference for 8% N_2O was statistically significant [mean percent time in the clear chamber types was 58.5 (N_2O-clear) versus 32.6 (N_2O-striped), $P = .042$]. The effect of N_2O on activity was evaluated in the control rats that received N_2O for the first time during the second test session. Activity levels were reduced by about 75%, from a mean of 26.7 crossings between chambers in the control group during the first (placebo) session to a mean of 6.7 crossings in control group animals exposed to 60% N_2O during the second test

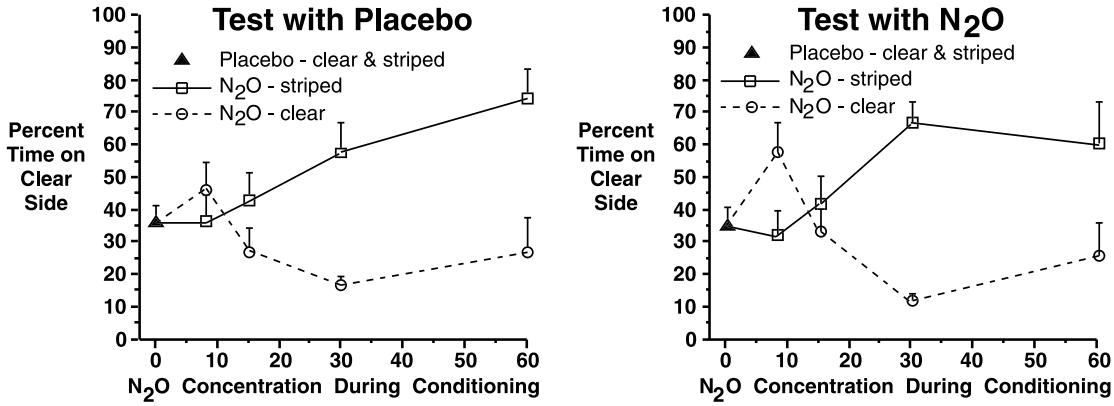

Fig. 1. During both placebo (left panel) and N_2O (right panel) test sessions, rats exhibited a conditioned place aversion to environments previously paired with 30% and 60% N_2O. A conditioned place preference was observed during the N_2O test session for the environment previously paired with 8% N_2O. The mean and S.E.M. are provided.

session (P=.001 by paired t test). No statistically significant effects on activity were observed at 8%, 15%, or 30% N_2O.

5. Discussion

Rats acquired a conditioned place aversion for the environment previously paired with 30% and 60% N_2O. This aversion did not depend on whether the test session took place in the absence or presence of N_2O and thus ruled out a state-dependent memory effect. The baseline preference for the striped chamber over the clear chamber may have made it difficult to demonstrate an increasing aversion to the clear chamber with increasing N_2O concentrations because of a floor effect in the possible amount of time reduction in the clear chamber. Fortunately, the bias in mean preference for the striped chamber versus the clear chamber was small (63.2% time in the striped chamber versus 36.8% time in the clear chamber) and did not threaten the interpretation of the results. Nevertheless, it would be ideal to better equate the baseline preference for the two distinct place conditioning environments. An initial exposure to 60% N_2O caused a reduction in locomotor activity in the control group. N_2O has been previously shown to reduce locomotor activity in rats and acute tolerance develops to this effect during a prolonged exposure (Dzoljic et al., 1994).

Although there are few data available on the reinforcing effects of N_2O, monkeys self-administer 60% N_2O (Wood et al., 1977) and humans self-administer 20%, 30%, and 40% (maximum dose tested) N_2O in an experimental situation (Walker and Zacny, 2002). In the present study, rats developed a conditioned place aversion to concentrations of 30% and 60% N_2O, which may seem surprising considering the human and monkey data that suggest these N_2O concentrations are reinforcing. Of course, caution must be used when extrapolating findings from research done in different species using different methods. For example, the statistically significant CPP for 8% N_2O during the second test session might suggest that rats experience the rewarding effects of N_2O at lower concentrations than do monkeys or humans. Additionally, drugs that support self-administration behavior do not always cause a CPP. In their critical analysis of the place conditioning paradigm, Bardo and Bevins (2000) concluded that despite a reasonable concordance in the rat literature indicating that drugs that are self-administered usually produce CPP, there are also notable exceptions to this general observation. The goal of Experiment 2 was to develop a method to assess N_2O self-administration in the rat.

6. Experiment 2a: a rat model of N_2O self-administration: the alternating gas environment paradigm

Like other inhalants, N_2O has the complexities associated with delivering a well-controlled "dose" of the drug within the constraints of a self-administration paradigm. However, N_2O has some unique and convenient characteristics that facilitate the development of a self-administration method. For example, N_2O's low solubility in blood and tissues means that a steady-state concentration can be quickly achieved and easily maintained, and that elimination of the drug is also rapid. Equilibration of the alveolar concentration with the inspired concentration of N_2O approaches 90–100% within 10–15 min (Eger, 1985). In addition, N_2O equilibrates more quickly in vessel-rich tissues like the brain, heart, and endocrine glands than in less-vascular tissues like fat and muscle. This rapid equilibration in the brain is thought to explain the quick onset of the drug's centrally mediated effects. Once equilibration has occurred, measuring the concentration of N_2O in the animal is simple, because it equals the concentration of N_2O in the chamber which can be measured continuously with infrared spectroscopy. There are no metabolic path-

ways for N_2O, although it was estimated that 0.004% of the N_2O taken up during an administration may be metabolized by anaerobic intestinal bacteria (Trudell, 1985). Thus, any contribution of dispositional (i.e., pharmacokinetic) factors to explain changes in the effect of N_2O over time is minimal. However, N_2O is known to inactivate cobalamin-dependent methionine synthase (Drummond and Matthews, 1994a,b), and whether inactivation of this enzyme may influence a dependent measure during a chronic experiment is unclear.

Our initial objective was to construct separate gas exposure chambers containing different concentrations of N_2O. These chambers needed to be gas tight and yet allow the rat free access to move between the chambers. The clear polycarbonate gas exposure chambers described in the place conditioning experiment were used as the exposure chambers for the self-administration experiments. However, the chambers were now connected by gas-tight, bidirectional "doors" that prevented mixing of the gases between the chambers. A rat could easily open a door by gently pushing on it and thus was able to move between chambers. The gases in the two adjacent chambers mix when a door opens, but a continuous inflow of gas to each chamber reestablishes the concentrations once the door automatically returns to its closed position. The amount of time a rat was in a specific N_2O concentration was measured as an index of its self-administration behavior. Preliminary tests placed one rat at a time in a linear gradient of four chambers connected by three gas-tight door assemblies. Each chamber contained a different concentration of N_2O (i.e., 0%, 20%, 40%, and 60% N_2O), and the length of each chamber was sufficient so that a rat could not open two doors simultaneously. Preliminary tests revealed that it was difficult to infer motivated drug-seeking behavior when a rat would spend considerable time in a specific chamber. Therefore, the polarity of the sequential order of drug concentrations within the gradient was reversed during a test session to see if the rat would change locations to seek out its preferred N_2O concentration. Preliminary data were encouraging, and a simpler two-chamber alternating gas environment procedure was investigated.

7. Material and methods for Experiment 2a

7.1. Subjects

Four adult male Long–Evans rats (Simonsen Laboratory) weighing approximately 200–250 g at the start of the experiment were studied. All rats were individually housed in a temperature-controlled ($\sim$23 °C) colony room with a 12:12-h light–dark cycle (light cycle from 7:00 a.m. to 7:00 p.m.). Rat chow and water were available ad libitum. The Institutional Review Committee for the use of Animal Subjects approved the procedures used in this study, and the research was conducted in an AAALAC-approved facility.

7.2. Apparatus

The gas exposure chambers were identical to the clear ones used in the place conditioning experiment. The gas delivery unit was slightly modified to allow alternation of the placebo and the N_2O between the two chambers via computer-controlled solenoid valves (Parker Hannifin). The infrared gas analyzer serially sampled the N_2O concentration in the exhaust gas from each of the four chambers approximately once every 2 min.

The doors connecting the chambers were designed to seal tightly when closed. The shape of the door had to allow it to be opened sufficiently so that the rat could pass through easily. A "double-hung" door design (i.e., where one door is hung inside the other) permitted the rat to move through the door in either direction. Magnets were placed in the doors to close them completely. The door assembly was fabricated out of aluminum, which was then anodized. Infrared optical sensors were used to determine where the rat was located within the apparatus.

7.3. Procedure

Rats were trained to operate the door assembly. Two chambers were connected by a door assembly and a plunger-like device consisting of a round polyethylene disk (diameter = 11.5 cm) with a handle was placed in each open end. These plungers were used to train the rats to move through the door. In brief, a rat was placed in a chamber and the plunger was moved forward so that the rat was limited to a small distance of approximately 7.5–10.5 cm. The rat would eventually lean against the door which would then open and allow the rat to enter the other chamber where the plunger was retracted the full distance. Using this training technique, a rat would pass through the door approximately 25 times during a 1-h training session each weekday. Training took place over a 2-week period until the rats could move easily through the doors.

During self-administration testing, a rat was placed in a chamber, and the apparatus was sealed. Gas flow rates were 2 l/min into each chamber. The concentrations of N_2O used to investigate drug-seeking/avoidance behavior were selected based on the N_2O concentrations that had an obvious effect in the place conditioning experiment. A concentration of 60% N_2O was used in all sessions except for several sessions where 30% N_2O was delivered (sessions 33–37 for Rats 1 and 2; sessions 32–34 for Rats 3 and 4). The side that received the N_2O first was determined randomly by the computer at the start of each 3-h test session. The rate at which the gas alternated between each segment was under computer control. Five alternations of 36-min periods were used initially (i.e., sessions 1–12), but this was reduced to four alternations of 45-min periods for the remainder of the test sessions. Self-administration sessions were conducted in a dark room, although two of the rats had a dim, flashing (50% cycle at 1 Hz) white light LED located

beneath the chamber receiving N_2O, while the other two rats had the flashing LED below the chamber receiving placebo. Two self-administration units could be operated simultaneously, which made it possible to test up to four rats each weekday between 9:00 a.m. and 5:00 p.m.

7.4. Data analysis

Within a session, drug self-administration behavior would result in a rat seeking out (i.e, spending more time in) the chamber with N_2O. A rat avoiding N_2O would spend more time in the chamber containing placebo. Rats with no preference or aversion to N_2O as well as rats unable to learn the self-administration contingency would show no chamber preference as a function of N_2O concentration. By knowing the rat's position in the apparatus and the N_2O concentration in each chamber of the apparatus, it was possible to reduce the data to the mean N_2O concentration a rat received during a session. The maximum concentration a rat could obtain during a 3-h session was also calculated as the average of the higher of the two N_2O concentrations in either chamber at each gas-sampling interval during the 3-h session. Thus, it was possible to calculate the percent of the maximum possible N_2O exposure a rat actually received during each session.

8. Results

Fig. 2 illustrates the N_2O self-administration behavior and the amount of movement between chambers exhibited by the four rats over repeated test sessions. Rats 1 and 2 appeared to choose the chamber based on the N_2O concentration as is apparent on selected test sessions (Fig. 3A–D). Rat 3 seemed to initially avoid N_2O (e.g., Fig. 3E) but then eventually stopped moving between chambers (Fig. 2). Rat

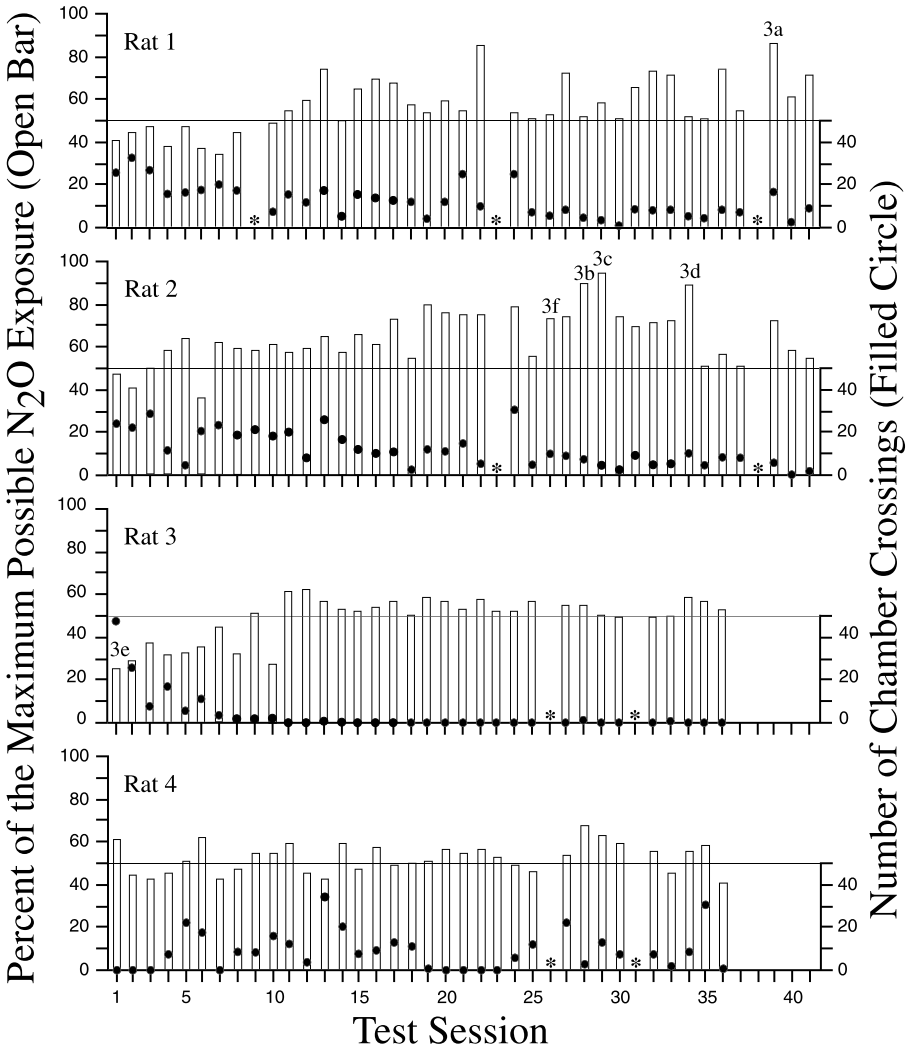

Fig. 2. The open bar indicates the percent of the maximum possible N_2O exposure for each rat during all sessions in the alternating gas environment paradigm. The filled circle indicates the number of chamber crossings. An asterisk indicates missing data, and the number written above some open bars indicates that the data collected during that session are shown in detail in Fig. 3.

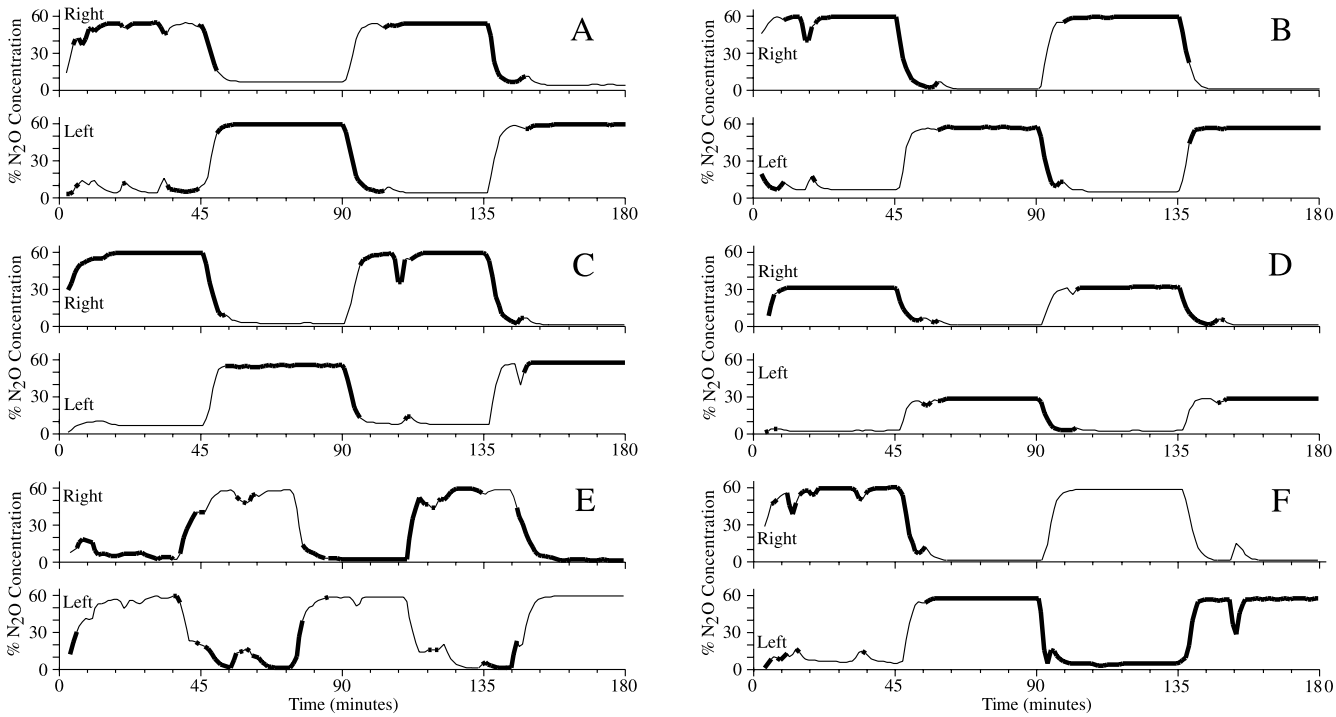

Fig. 3. A rat's choice of location varies as a function of N_2O availability as illustrated during selected single sessions in the alternating gas environment paradigm. The bolded portions of the N_2O concentration curves indicate the location of the rat within the apparatus as well as the concentration of N_2O the rat is breathing. Panels: A (Rat 1, session 39), B–D (Rat 2, sessions 28, 29, and 34, respectively), E (Rat 3, session 1), F (Rat 2, session 26). Brief chamber crossings are not visible in this graphical representation of the data.

4 did move between chambers, but its choice of chamber did not suggest that it was seeking or avoiding N_2O (Fig. 2).

9. Discussion

A reversal design (e.g., ABAB) is a type of single-subject experimental design that investigates whether an experimental intervention controls a behavior. Data obtained from these designs can be convincing when a reliable temporal connection exists between the manipulated variable and the target behavior. Indeed, the N_2O self-administration data illustrated in Fig. 3B–D argue strongly that Rat 2 would sometimes move within the apparatus as a function of where the N_2O was available. However, there were numerous sessions where the interpretation of the self-administration data was less obvious. For example, during some sessions, a rat would appear to exhibit drug-seeking behavior but then would remain in a chamber despite the switch from N_2O to placebo gas (e.g., Fig. 3F). In such situations, the reversal design requires the interpretation that the rat now prefers placebo gas to N_2O by virtue of its remaining in the chamber with the placebo gas. This resembles a "forced-choice" procedure, because a rat's self-administration behavior must be classified as either preferring N_2O or preferring the placebo gas. Recent human research (Walker and Zacny, 2002) describes how a forced-choice procedure can make it more difficult to demonstrate the reinforcing effects of N_2O than a "free-choice" procedure. In contrast to a subject being forced to choose either N_2O or placebo, the free-choice procedure allows the subject to choose between N_2O, placebo, or no drug (i.e., room air). Human subjects often report that they like N_2O, but that they also want to take breaks from the drug (Walker and Zacny, 2002). When a subject is only allowed to choose between N_2O and placebo, these breaks are interpreted as a placebo preference. The free-choice procedure allows subjects to take breaks from the N_2O administration by selecting the no-drug option rather than by choosing to administer the placebo gas. Thus, the free-choice procedure can magnify the discrepancy between the self-administration of placebo versus N_2O and this results in a more sensitive measure of the drug's reinforcing effects. The goal of Experiment 2b was to evaluate a rat model of N_2O self-administration using a free-choice procedure.

10. Experiment 2b: a rat model of N_2O self-administration: the free-choice procedure

A standard rat housing tub was modified by adding two gas exposure chambers such that one chamber was protruding at a right angle from each side of the tub. The tub had the typical wire lid that was open to room air and that held food and a water bottle. The gas chambers were positioned

directly opposite one another and were connected to the tub via the gas-tight door assembly that fit over a machined collar glued to the right and left sides of the tub. This design of the self-administration apparatus allowed the rat to choose between room air (with access to food and water), placebo gas, or N_2O.

11. Material and methods for experiment 2b

11.1. Subjects

The same four rats that had been studied in the previous self-administration experiment were evaluated using this new procedure. Each rat was tested individually and was placed in the central portion of the free-choice apparatus where rat chow and water were available ad libitum. The light cycle in this testing room had the lights on from 7:45 a.m. to 7:45 p.m. The Institutional Review Committee for the use of Animal Subjects approved the procedures used in this study, and the research was conducted in an AAALAC-approved facility.

11.2. Apparatus

A wire grid floor was placed in the tub above the bedding material to prevent wood chips from blocking the closure of the gas-tight doors. The gas exposure chambers protruding from the right and left sides of the tub were identical to the clear ones used in the place conditioning. The infrared gas analyzer serially sampled the N_2O, oxygen, and carbon dioxide concentrations in the exhaust gas from each of the two chambers approximately once every minute. An infrared optical sensor array detected when the rat was in either the N_2O or placebo chambers.

11.3. Procedure

Test sessions began in the afternoon by placing the rat in the central chamber of the apparatus. The session ended the next afternoon when the rat was removed from the apparatus and placed in a holding tub for approximately 1 h. Between sessions, the apparatus was cleaned, the rat was weighed, and the gas tanks were replaced as needed. In this study, the gas flow rate into each chamber was 1 l/min to ensure an adequate gas supply. The chamber that received 60% N_2O was counterbalanced between the right and left sides over each rat's test sessions. A dim, white light LED flashed (50% cycle at 1 Hz) above the door assembly leading to the chamber containing N_2O. Rat 2 was tested initially, because it showed the clearest self-administration behavior in the alternating gas environment paradigm. Rat 2 was tested for 12 consecutive sessions, while the other three rats received six sessions each. After all rats had been tested, Rat 3 was given six additional sessions to clarify whether it was reliably avoiding N_2O.

11.4. Data analysis

During each session, a rat's presence in either gas exposure chamber (i.e., the N_2O or placebo chamber) was recorded in real time using infrared optical detectors. This information was used to calculate two dependent measures: (1) the total number of entries a rat made into each of the gas exposure chambers from the central room air chamber, and (2) the total duration of time a rat spent in each chamber. The rat's time in the central tub was calculated by subtraction of the time spent in either of the gas chambers from the total length of the session. Individual sessions were categorized according to whether there was a preference for N_2O (greater entries or longer duration in the N_2O chamber than the placebo chamber), an aversion to N_2O (fewer entries or shorter duration in the N_2O chamber than the placebo chamber), or no preference/aversion to N_2O (equivalent entries or duration in the placebo and N_2O chambers). Sign

Table 1
Self-administration data collected from each rat during the free-choice procedure

Animal	Session	Side with N_2O (left or right)	Minutes in chamber			Number of chamber entries	
			N_2O	Center	Placebo	N_2O	Placebo
Rat 1	1	L	815	203	331	114	58
	2	R	884	205	330	58	40
	3	L	1131	212	68	42	14
	4	R	730	198	483	31	26
	5	L	865	191	359	28	13
	6	R	1104	173	120	40	23
Rat 2	1	L	802	321	11	105	12
	2	R	594	350	446	129	66
	3	L	1179	201	19	132	12
	4	R	855	257	292	263	64
	5	L	912	320	190	333	108
	6	R	1144	246	15	268	8
	7	L	682	400	316	91	94
	8	R	978	363	78	113	20
	9	L	758	640	8	33	12
	10	R	1039	300	67	128	12
	11	L	926	410	86	71	28
	12	R	1073	333	11	78	4
Rat 3	1	R	0	1426	0	0	0
	2	L	65	982	267	2	78
	3	R	0	1283	0	0	0
	4	R	0	1116	261	0	3
	5	L	25	1138	216	4	10
	6	L	0	763	606	0	23
	7	R	130	594	692	6	29
	8	L	89	185	1109	12	22
	9	R	213	270	884	24	105
	10	L	24	618	754	8	58
	11	R	252	1118	0	14	0
	12	L	26	416	917	2	20
Rat 4	1	L	0	1299	30	0	2
	2	R	461	897	3	92	4
	3	L	0	1338	0	0	0
	4	R	0	1309	0	0	0
	5	L	0	1354	0	0	0
	6	R	0	1351	1	2	2

tests were used to determine whether each rat exhibited a reliable preference or aversion to N_2O over multiple sessions. Separate tests were performed for number of entries and total duration of time in each chamber. The sign test was based on the exact binomial distribution for the proportion of sessions that showed a preference for a specific gas; ties were excluded (i.e., sessions that did not show a preference or aversion to N_2O). Two-tailed significance levels were reported.

12. Results

Table 1 summarizes the self-administration data collected from the four rats during the free-choice test sessions. Rat 1 preferred the chamber containing the N_2O to the chamber containing the placebo in all six test sessions (sign test, $P < .05$). Results from the sign tests for this rat were identical using the number of chamber entries and total duration in each chamber. Rat 2 also exhibited a clear preference for N_2O; this rat entered the chamber containing N_2O more frequently than the chamber containing placebo in 11 of 12 test sessions (sign test, $P < .001$) and spent more time in the chamber with N_2O than in the chamber with placebo in all 12 test sessions (sign test, $P < .001$). Rat 3 avoided N_2O in 9 of 10 sessions in which any preference was shown (sign test, $P < .05$); the results were identical for number of chamber entries and duration in each chamber. Rat 4 did not appear to have a preference or aversion to N_2O (sign test, $P > .05$, using number of chamber entries or duration in each chamber); this animal spent most of the time in the central tub. There was no obvious circadian pattern of N_2O self-administration for Rat 1 or Rat 2. However, both of these rats did spend less time in the N_2O chamber during the first 2 h after being placed/returned to the apparatus than any other time during a session.

13. Discussion

The free-choice procedure was an effective strategy to investigate the reinforcing effects of N_2O. Data collected in the alternating gas environment paradigm suggested that Rat 1 and Rat 2 (Figs. 2 and 3A–D) found 60% N_2O reinforcing, and this conclusion was demonstrated conclusively using the free-choice procedure. The converse situation was also supported. Rat 3 seemed to initially avoid N_2O in the alternating gas environment paradigm (Fig. 3E), and this N_2O avoidance was confirmed with the free-choice procedure. Rat 4 did not show reliable self-administration behavior in either the alternating gas environment paradigm or the free-choice procedure.

Although the chamber duration measure and the chamber entry measure gave similar results in this study, they are not equivalent in their interpretation. Dependent measures that use the amount of time spent in a drugged environment are potentially confounded by a drug's effect on performance. For example, if a drug were to sedate an animal or otherwise makes it less able to leave the drugged chamber, this would inflate the amount of time spent in the drugged chamber relative to the placebo chamber for a reason unrelated to a motivation to self-administer the drug. In contrast, because entries into the drugged chamber or the placebo chamber are always made from a central area containing room air, the subject's choice of which chamber to enter cannot be attributed to a direct effect of the drug on performance. Rather, a subject's reliable preference to choose to enter either the placebo or N_2O chamber indicates a differential motivation to seek (or avoid) the drug.

The free-choice procedure has several advantages for studying N_2O self-administration. This method requires little direct involvement from the investigator (e.g., less than an hour per day), experimental control and data acquisition are automated, and the reinforcing effects observed in Rat 1 and Rat 2 were unambiguous. It is unclear whether the prior experience in the alternating gas environment paradigm was necessary for these two rats to show N_2O's reinforcing effects in the free-choice procedure. The alternating gas environment paradigm initially gave all rats comparable N_2O exposure which is an important consideration when studying individual differences in the acquisition of drug self-administration (Piazza et al., 1998). This comparable drug exposure probably would not occur for rats placed initially in the free-choice procedure because individuals who are less likely to explore may not leave the center chamber and thus would not be exposed to the N_2O in one of the side chambers. It is also the case that the alternating gas environment procedure may better identify rats that avoid N_2O, because this paradigm requires a rat to behave to avoid the drug. This is not the case in the free-choice procedure where the absence of behavior (i.e., remaining in the center chamber and not entering either side chamber) complicates the interpretation of N_2O avoidance.

The self-administration paradigm described here is novel and additional research will be needed to determine the optimal method by which rats are trained to self-administer N_2O. Although only four rats were evaluated for N_2O self-administration, it is clear that two rats acquired the self-administration behavior. However, the additional finding that one rat avoided N_2O suggests that there may be meaningful individual differences in N_2O's reinforcing effects. Like humans, animals exhibit individual differences in their propensity to self-administer drugs (Piazza and Le Moal, 1996). Animals that do not self-administer a drug or do not provide a stable baseline level of drug self-administration are often excluded from experiments involving drug self-administration (Woods, 1998). Additional research will be needed to better estimate what proportion of rats will self-administer N_2O and whether individual differences in drug-taking propensity can be predicted.

14. General discussion

Inhalant abuse has been described by Balster (1997, p. 3) as "A Forgotten Drug Abuse Problem" which, despite its importance, "has been neglected by the vast majority of drug abuse scientists." There are undoubtedly many factors that have led to this situation, but the difficulty associated with adapting certain behavioral pharmacology methods for use with inhalants has contributed to the problem. For example, good progress has been made in understanding the neural mechanisms underlying N_2O's analgesic and antinociceptive effects (Fujinaga and Maze, 2002; Quock and Vaughn, 1995) where rodent pain assessment methodologies have been available for use with N_2O. In contrast, nothing is known about the neural basis of N_2O reinforcement or reward with the exception that 1 mg/kg naloxone does not alter the reinforcing effects of 67% N_2O in monkeys (Grubman and Woods, 1982). The development of self-administration and place preference paradigms for N_2O that can be used with rats should facilitate research on the neuropharmacology and neurobiology of N_2O abuse.

Concentrations of N_2O that caused a conditioned place aversion were also self-administered in the present study. In the rat literature, drugs that cause CPP are usually self-administered as well, and thus, N_2O is atypical in this regard. However, like N_2O, ethanol causes a conditioned place aversion in rats (Bormann and Cunningham, 1998; Cunningham, 1981) and yet is self-administered. This finding adds to the growing list of behavioral and pharamacological similarities between N_2O and ethanol (Johanek et al., 2001; Kaiyala et al., in press).

Our inhalant self-administration methodology has several strengths that may make it attractive when investigating questions about drug addiction that do not necessitate the use of a specific drug. By using an inhalant, this method does not require the animal to undergo any surgery to enable drug self-administration. Thus, the labor-intensive process of placing and maintaining cannulae to allow intravenous or intracerebral delivery of a drug is eliminated. Furthermore, we believe the apparatus can be reduced in size to accommodate inhalant self-administration studies in mice where these surgical procedures are considerably more complex. Because the experimental procedures and data acquisition are automated, it should be possible to test large numbers of rats or mice simultaneously. The efficiencies offered by this new methodology may make it an appealing approach for drug self-administration research.

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