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An adjusting-dose procedure for assessing the reinforcing effects of nitrous oxide with humans ☆

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ABSTRACT

Despite continued abuse, there is a paucity of empirical investigations on inhalants as reinforcers. The present study attempted to derive a method for studying the reinforcing effects of nitrous oxide (N_2O) with human participants. An adjusting-dose procedure was employed to assess choice allocation for inhalation periods of varying doses of N_2O. After experiencing the experimental parameters in forced-choice trials, participants made choices between a fixed dose of 0% N_2O (i.e., 100% O_2) and an adjusting dose of N_2O (0–50% N_2O in O_2). The adjusting dose titrated as a function of the participant's choices. Conditions were run to stability and systematically replicated within-subject. Stable choice allocation served as both the chief dependent variable and an indication of the optimal reinforcing dose of N_2O for that participant. Consistent with previous research on N_2O, there was between-subject variability in the reinforcing effects of N_2O; however, stable within-subject choice allocation was observed for 6 out of 8 participants. This method of assessing drug choice in humans allows for the testing of multiple doses within-subject, which is imperative, given that the reinforcing effects of drugs are known to vary across subjects and as a function of dose.

1. Introduction

Nitrous oxide (N_2O) is a gas at room temperature and pressure and is primarily used as an anesthetic for minor to moderate oral surgery, but also as a propellant for whipped cream, and an agent to boost octane levels in racing cars (U.S. Dept. of Health and Human Services, 2000). Despite continued self-administration of N_2O for nonmedical purposes, inhalant abuse has received much less attention from the scientific community relative to other abused drugs (Balster, 1998). One issue imperative to gaining a greater understanding of the abuse potential of N_2O is an exploration of the reinforcing effects of the drug.

Researchers have investigated dose–response relations during administration of N_2O with humans under choice procedures in an attempt to better understand the reinforcing effects of N_2O. In a study examining the reinforcing effects of N_2O using a choice procedure, Walker and Zacny (2001) assessed the choices of 12 participants with no history of drug dependence. In each of five sessions, after sampling 30% N_2O ("Agent A") and 100% O_2 ("Agent B") for 10 min each, participants then chose nine times, once every 5 min, among the two sampled agents or drug-free air (i.e., "neither"). An analysis that combined all participants' choices (540 choice trials) revealed that N_2O was chosen on 41% of the trials, 100% O_2 (placebo) was chosen on 11% of the trials, and "neither" was chosen on 48% of the trials. An analysis of individual participants' data, however, show high between-subject variability in the proportion of N_2O choices, but similar choice allocation was observed within-subject across sessions in 10 out of the 12 participants, indicating within-subject stability in choice allocation.

The focus of the Walker and Zacny (2001) study was an analysis of within- and between-subject variability in choice under the effects of the same dose of N_2O administered on different days; therefore, participants were tested with only one dose of N_2O (i.e., 30%). It is important to note, however, that several studies have suggested that reinforcing effects of a drug vary as a function of dose (e.g., Balster and Schuster, 1976). In a subsequent experiment, Walker and Zacny (2002) replicated the study described above, using a range of doses. The reinforcing effects of five doses of nitrous oxide (0, 10, 20, 30, and 40% N_2O) were analyzed across five sessions. Twenty participants were exposed to a particular dose of N_2O ("Agent A") for 10 min and 100% O_2 ("Agent B") for 10 min. They were then asked to choose among Agent A, Agent B, and "neither." Dose order was randomized across participants. The results indicate that, in general, mean N_2O choice increased with increasing N_2O dose, but there was, again, between-subject variability. The authors analyzed each participant's choice allocation to get an

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indication of an optimally reinforcing dose for that participant. For example, during the 5 sessions, Participant S-5 chose 0% N_2O two times, 10% N_2O five times, 20% N_2O eight times, 30% N_2O five times, and 40% N_2O four times. This participant's choice allocation suggests that the optimal reinforcing dose of N_2O for that participant was 20%. Although Walker and Zacny (2002) tested multiple doses, which allowed for the analysis of dose-dependent effects within-subject, each participant was only exposed to one dose per session, with no replication of any dose. This procedural limitation precluded the opportunity to assess whether a participant's choice allocation for inhalation periods of a particular dose of N_2O would remain consistent across sessions.

In an attempt to assess the reinforcing effects of a wide range of N_2O doses while allowing for exposure to multiple doses within a session, the present study employed an adjusting-dose procedure. Adjusting (also called titration) procedures have been employed as a means of adjusting variables of interest as a function of the organism's choice allocation. Two variables that are often adjusted are delay to reinforcement (e.g., Mazur, 1987) and amount of reinforcement (e.g., Richards et al., 1997).

Mazur (1987) introduced an adjusting-delay procedure useful for the analysis of choice. In this study Mazur was interested in identifying indifference points between smaller reinforcers delivered sooner and larger reinforcers delivered later. Pigeons made repeated choices between pecking a key that resulted in 2 s access to grain after a fixed delay, and pecking a key that resulted in 6 s access to grain after an adjusting delay that increased or decreased depending on the pigeon's local choice patterns. Choice trials occurred in blocks of four. Each trial block had two forced-choice trials in which only one key was illuminated and operative. This allowed the pigeon to be exposed to the current delay values on each alternative during each trial block. The two forced-choice trials were followed by two free-choice trials in which both the previously presented alternatives were available concurrently. If the pigeon chose the larger reinforcer (6-s access) on both free-choice trials, the delay to the larger reinforcer on the subsequent trial block was increased by 2 s. If the pigeon chose the smaller reinforcer (2-s access) on both free-choice trials, the delay to the larger reinforcer was decreased by 2 s. Eventually, the pigeon's choice allocation stabilized the adjusting delay to the larger reinforcer around what Mazur called an indifference point, that is, a delay where the subjective value of the 6-s reinforcer equaled that of the 2-s reinforcer.

Mazur's (1987) adjusting-delay procedure introduced the methodological framework for using titration procedures in the study of choice; however, a more direct analogue of the procedure employed in the present study is an adjusting-amount procedure. Richards et al. (1997) used an adjusting-amount procedure to investigate reinforcer delay-discounting functions with rats. Instead of adjusting the delay to reinforcement, adjusting-amount procedures adjust the reinforcement amount for one of the alternatives. In this study, rats made choices between a larger fixed amount of water delivered after a delay, and an adjusting amount of water delivered immediately. The amount of immediately delivered water was adjusted as a function of the rat's response allocation. Indifference points were derived from the values at which the rat chose the immediate amount of water and the delayed amount of water with equal frequencies.

The present study employed the structure of Mazur's (1987) 4-trial block component with an adjusting-amount (dose) procedure (cf. Richards et al., 1997) to identify the optimal reinforcing dose of N_2O in human participants. The optimal reinforcing dose was defined in the present investigation as the dose at which choice allocation to the two options stabilized for each participant.

2. Methods

2.1. Participants

The study was approved by the local Institutional Review Board and was conducted in compliance with the Declaration of Helsinki. Eight participants (5 females and 3 males, age 21–29 [mean age = 24.5]) were recruited via poster and newspaper advertisements. Prospective participants were screened in accord with existing laboratory protocols. Specifically, they completed the SCL-90, a questionnaire designed to assess psychiatric symptomatology (Derogatis et al., 1973), the Michigan Alcoholism Screening Test (Selzer, 1971), and a health questionnaire designed to determine medical, psychiatric, and drug-use history. Potential participants were excluded if they had a history of Axis-I psychiatric disorders, including drug- or alcohol-related problems, as defined by the Diagnostic and Statistical Manual of Mental Disorders-IV (American Psychiatric Association, 1994); if they tested positive for amphetamines, barbiturates, benzodiazepines, cocaine metabolites, opiates, or phencyclidine; if they had experienced any adverse reactions to general anesthetics; if they had pulmonary, renal, hepatic, or cardiac disorders; or if they reported *no* recreational use of alcohol or other drugs.

Participants who met the inclusion criteria attended an orientation session during which they signed a written consent form that described the study. The consent form stated that the drugs to be used in this study were drugs commonly used in medical settings and could come from one of six classes delivered via gaseous or aerosol form — sedative/tranquilizer, stimulant, general anesthetic (at subanesthetic doses), opiate, alcohol, or placebo. In addition, at the orientation session they underwent a resting-state electrocardiogram, a medical examination, gave a urine sample for drug toxicology screening, and were fitted for an anesthesia mask.

Payment for study participation ($57 per session) was made at a debriefing session at least 24 h after the last experimental session. Payment was not dependent on session performance, only session attendance.

2.2. Apparatus and setting

The experimental room consisted of a reclining chair, a television, an anesthesia machine, and resuscitative equipment. The anesthesia machine (North American Drager Narkomed®) was located behind the recliner. All parts of the machine that might identify the gases delivered were covered so that the participant and the research technician remained blind to the agents being administered. Inhalation periods were controlled by an anesthetist seated behind the participant, next to the anesthesia machine. The research technician sat at a desk about 1 m away from the recliner. To better approximate a naturalistic setting, participants could engage in leisure activities while seated in the reclining chair (e.g., watching movies or television, reading, and listening to music). School- or work-related activities were not permitted because previous research has shown that these activities can influence the reinforcing effects of drugs (e.g., Comer et al., 1996; Silverman et al., 1994).

2.3. Procedure

Upon arrival to the laboratory, participants signed a compliance form stating that they had abstained from eating for 4 h prior to the session and drinking for 2 h prior to the session, as well as alcohol or other drugs (excluding caffeine and nicotine) for 24 h prior to the session. If they had engaged in any of the activities above too close to the session time, the session was rescheduled. Prior to each session, participants delivered a breath sample, which was analyzed for the presence of alcohol by a breath intoximeter (Alcosensor-3, Intoximeters, Inc., St. Louis, MO). In addition, female participants gave a urine sample for a pregnancy test, and negative results were required before the session could begin. Participants were also told that they could be tested for the drugs listed above before any or all sessions, and positive results would result in termination from the study. On the third session, participants delivered a urine sample for toxicology screening.

Participants were seated in the recliner and fitted with a blood pressure cuff, pulse oximeter, and anesthesia mask. The anesthetist

delivered 100% O_2 through the mask, while the research technician told the participant, "You are now breathing drug-free air." After the participant was breathing properly through the mask, the session proper began.

Each 3-hour session was divided into three 40-min trial blocks followed by a 60-min recovery period. Participants sampled and then chose between an adjusting dose of N_2O and a fixed dose of 0% N_2O (i.e., 100% O_2). We used 100% O_2 rather than compressed air, because O_2 was the vehicle and because previous research found no differences in psychomotor performance or subjective effects between 100% O_2 and compressed air (e.g., Dohrn et al., 1992). Choices were made by circling the printed words 'Choice 1' or 'Choice 2' on a sheet of paper presented to the participant on a clipboard by the research technician. Whether 'Choice 1' referred to the adjusting dose or the fixed dose was counterbalanced across participants. Each trial block consisted of 2 forced-choice trials and then 2 free-choice trials. Each choice trial was 5 min long and was followed by a 5-min intertrial interval (ITI), in which the participant was administered "drug-free air" (i.e., 100% O_2). The ITI served as a washout period, and the duration of the ITI was determined by previous research showing no or very low concentrations of N_2O in the participants' expired air and participants' verbal reports indicating little to no effect of drug after 5 min.

The research technician read the following instructions to the participant prior to the first trial block of the first session:

> During each session, you will be making responses by circling one of the choices on the page given to you. Sometimes you will be presented with one choice. During these trials, please circle the only available choice. Sometimes you will be presented with two choices. During these trials, please choose one of the options. The choice is entirely up to you.

During the 2 forced-choice trials, only one choice was printed on the paper presented to the participant. After the participant chose the only available option, the technician informed the participant: "You are now breathing 'Choice 1' ('Choice 2') for the next 5 min." The anesthetist then delivered the chosen dose of N_2O. The function of the forced-choice trials in each trial block was to help ensure that the participant had continuing exposure to the adjusting contingencies. At the beginning of each free-choice trial, a sheet of paper with two choices (i.e., 'Choice 1' and 'Choice 2') was presented to the participant. After the participant chose one of the two options, the technician informed the participant: "You are now breathing 'Choice (1 or 2)' for the next 5 min." Again, the anesthetist delivered the chosen dose. After every forced- and free-choice trial, the anesthetist began the intertrial interval by administering 100% O_2 as the technician informed the participant: "You are now breathing drug-free air for the next 5 min."

The adjusting dose titrated as a function of the participant's responses. For example, if Choice 1 (C1) was the adjusting dose and the participant chose 'Choice 1' on both free-choice trials of a trial block, the adjusting dose of N_2O (Choice 1) increased by 10% (i.e., C1 + 10%). If the participant chose 'Choice 2' (i.e., the fixed dose of 0% N_2O [100% O_2]) on both free-choice trials of a trial block, the adjusting dose of N_2O (Choice 1) decreased by 10% (i.e., C1 − 10%). If the participant chose 'Choice 1' on one free-choice trial and 'Choice 2' on the other, the adjusting dose (Choice 1) remained the same (i.e., C1 + 0%). A ceiling dose of 50% N_2O was implemented for all participants because data from this laboratory (Walker and Zacny, 2003) show that 50% N_2O can produce effects that may be aversive (e.g., nausea, vomiting, extreme dysphoria), and we did not want to expose our healthy volunteers to a higher dose, which may produce more aversive effects. It was this bitonic nature of N_2O observed by Walker and Zacny (2003) that led us to adopt the strategy of increasing (rather than decreasing) the adjusted dose if it was chosen on both free-choice trials. That is, although both Mazur (1987) and Richards et al. (1997) made the adjusting alternative less attractive when it was chosen exclusively (by increasing the delay to, or decreasing the amount of, that alternative), previous research has shown that N_2O dose preference is bitonic suggesting that as the dose approached high non-preferred levels, the fixed dose of 0% N_2O would be chosen thus adjusting it downward. The adjusting contingencies of this procedure, therefore, serve to identify concentrations that are too high (i.e., aversive) which would be indicated by preference for 0% N_2O.

There were two conditions in this study. In one condition, the adjusting dose began at 30% N_2O. In the other condition, the adjusting dose began at 10% N_2O. The order of conditions was counterbalanced across participants. The purpose of having a second condition was to attempt to replicate the observed adjusting dose in the first condition and to insure that the initial value of the adjusting dose was not responsible for the participant's choice allocation. Both conditions were run to stability. Our stability criteria consisted of three rules. First, a minimum of 3 sessions (i.e., 9 trial blocks) was required in the first condition to help insure sufficient exposure to the adjusting contingencies. Second, after the 3 sessions, each participant was required to maintain the adjusting dose at the same value or one of two adjacent doses for 5 consecutive trial blocks. Finally, there was a component of visual inspection in our criteria. If an overall upward or downward block-to-block trend was apparent, the participant remained in the condition for another session. The stability criteria for Condition 2 were identical to that described above with the exception of the 3-session minimum.

3. Results

Results for all participants are presented in Fig. 1. The value of the adjusting dose of N_2O is plotted across trial blocks. The first data point in each series indicates the initial value of the adjusting dose (i.e., the concentration of the adjusting dose of N_2O administered during the first trial block), and subsequent data points indicate the value of the adjusting dose inhaled during each subsequent trial block (the dose presented during forced-choice trials was always chosen by all participants, thus those choices are not represented in Fig. 1). The vertical dashed line indicates condition change.

Both Participants 2223 and 114 displayed similar patterns of responding, in that near-exclusive preference was observed for the adjusting dose of N_2O under both conditions. This choice pattern resulted in increasing the adjusting dose to 50% N_2O, and although both participants continued to choose the adjusting dose, as per our 50% ceiling contingency, the adjusting dose concentration remained at 50% N_2O. Participants 228 and 1212 both titrated the adjusting dose to moderate levels. Participant 228 reached stability with a 40% dose of N_2O in the first condition, and 20% N_2O in the second condition. Participant 1212 reached stability at 10% N_2O in the first condition and 10–20% N_2O in the second condition. Greater session-to-session variability was observed with Participant 142, however, this participant reached stability at near-zero and zero dose levels of N_2O in both Conditions 1 and 2, respectively. Participant 1813 had near-exclusive preference for the fixed dose of 0% N_2O. This choice pattern drove the adjusting dose of N_2O down to 0% in both conditions.

Failure to replicate choice allocation of adjusted dose concentrations between conditions was observed for Participants 1911 and 137. Both participants titrated their adjusting doses to 0–10% N_2O in the first condition and 50% N_2O in the second condition. Both of these participants were exposed to an initial dose of 10% in the first condition. It is possible, therefore, that these two participants did not contact the contingency of "choose adjusting dose twice, adjusting dose increases" in the first condition. In other words, the adjusting dose (initially set at 10% in the first condition) may not have been discriminable from the fixed dose (0%) and, therefore, may not have exerted control over the participants' behavior. In the second condition, however, the initial 30% dose appeared to be sufficiently salient to facilitate contact with the adjusting contingencies. If more sessions of exposure were available, a replication of the first condition

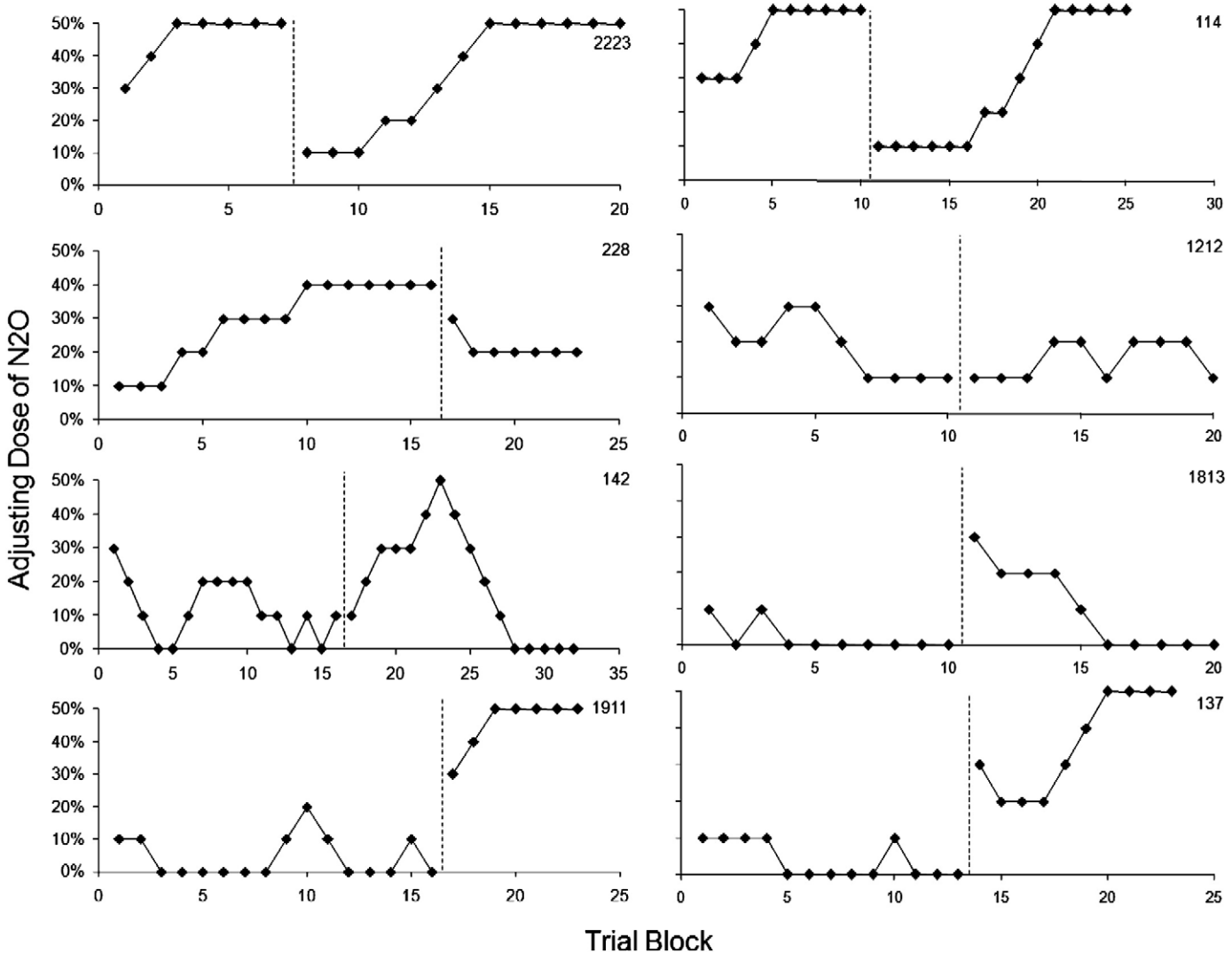

Fig. 1. The value of the adjusting dose of N_2O as a function of trial blocks for the eight participants. The first data point in each series indicates the initial value of the adjusting dose, and subsequent data points indicate the value of the adjusting dose inhaled during subsequent trial blocks. The vertical dashed line indicates condition change.

(i.e., initial dose of 10% N_2O) would have provided the means to experimentally evaluate the hypothesis above.

4. Discussion

Previous research has suggested that reinforcing effects of a drug vary as a function of dose (e.g., Balster and Schuster, 1976; Branch, 1991). It has also been suggested that exposure to multiple drug doses and multiple free-choice trials are useful when characterizing N_2O as a reinforcer (e.g., Walker and Zacny, 2001, 2002). The present procedure offered a methodological improvement to previous investigations of the reinforcing effects of N_2O by allowing for a wide range of doses to be assessed multiple times throughout each session of the study.

One advantage of this procedure is that, like other titration procedures, the programmed administration of independent variables is based on participants' performance instead of arbitrary (e.g., predetermined) sequences. Moreover, this procedure may allow for a more objective assessment of "preference." By providing sufficient exposure to adjusting contingencies, participants are allowed to adjust the doses of N_2O to desired levels, which provide evidence of the reinforcing effects of N_2O, for example, displayed in the choice allocation of Participants 2223 and 114, or a lack thereof, displayed in the choice allocation of Participants 142 and 1813. Furthermore, this procedure is especially useful when considering the individual differences in choice as a function of dose that have been observed when assessing N_2O in previous research.

In addition, the rapid-onset and short-acting nature of N_2O make it ideal for this procedure and allow it to serve as an immediately consumable reinforcer with human participants, analogous to Mazur's (1987) use of grain with pigeons and Richards' et al. (1997) use of water with rats. It is currently unclear, however, whether this method would elucidate the reinforcing effects of other drugs or other routes of administration. It is likely that the procedure would be useful for assessing other drugs with rapid onset and short duration of action, regardless of the route of administration (e.g., the ultra-short-acting intravenous opioid, Remifentanil). Its use with delayed-onset or longer-acting drugs would present a greater challenge, given the lesser effectiveness of delayed reinforcement (delayed onset) and the decreased number of trials possible in a session (longer duration of action). Future research on this topic will first require preliminary work to derive the appropriate drug-specific experimental variables. For example, studies conducting a parametric analysis of inhalation periods, adjusting step-size contingencies, ITI duration, session length, or selection of stability criteria, would serve to not only extend this procedure's generality, but also help identify possible variables responsible for the limitations of the present study, most notably, the within-subject variability. In addition, studies examining choice allocation between two different (non-zero) doses may further illuminate the reinforcing effects of the drug as well as the utility of this procedure.

This adjusting-dose procedure was designed to yield an empirical identification of the optimal reinforcing dose of N_2O with human participants. This procedure may have utility in future studies that plan to examine the determinants or modulators of N_2O's reinforcing effects. In addition to increasing our understanding of N_2O's reinforcing effects, this procedure could be extended to the study of other rapid-onset, short-acting drugs, such as other inhalants or drugs administered via the intravenous or intranasal route. Such studies may include this procedure as a preliminary experimental phase to determine which dose of drug should be used to reinforce responding most effectively and then, for example, conduct further manipulations to determine discounting functions and indifference points using the drug as an immediately consumable reinforcer.

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