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Lack of Acute Tolerance Development to the Subjective, Cognitive, and Psychomotor Effects of Nitrous Oxide in Healthy Volunteers

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YAJNIK, S., J. P. ZACNY, C. J. YOUNG, J. L. LICHTOR, G. RUPANI, J. M. KLAFTA, D. W. COALSON AND J. L. APFELBAUM. *Lack of acute tolerance development to the subjective, cognitive, and psychomotor effects of nitrous oxide in healthy volunteers.* PHARMACOL BIOCHEM BEHAV **54**(2) 501–509, 1996.—A crossover, double-blind trial was conducted using eleven healthy volunteers to determine whether and the degree to which acute drug tolerance occurred to the subjective, cognitive, and psychomotor effects of a range of subanesthetic nitrous oxide doses (0, 10, 20, 30, and 40%). There was little evidence of acute drug tolerance to the subjective measures or to the cognitive/psychomotor impairing effects of nitrous oxide at any of the concentrations tested over the course of the 120-min inhalation.

Acute drug tolerance Mood Nitrous oxide Cognition Humans Psychomotor Subjective General anesthetic

ACUTE tolerance is defined as a change of "sensitivity to a drug within the duration of one continuous drug exposure" (16). Possible mechanisms that have been postulated for the development of acute tolerance include alterations in levels of neurotransmitters or their receptors (18). Acute tolerance has been documented with a number of drugs including alcohol (24), nicotine (40), antihistamines (23), narcotics (17), benzodiazepines (13), and general anesthetics (17). The focus of the present study was on the inhalational anesthetic, nitrous oxide. The pharmacokinetic qualities of nitrous oxide make it an excellent agent for studying acute drug tolerance in that nitrous oxide has a low blood-gas partition coefficient (0.4), equilibrates quickly in the blood (12), and, once equilibrium is reached, remains at constant concentration levels as long as the gas is administered (12). Therefore, any lessening of nitrous oxide-induced drug effects over the time course of a single inhalation would be indicative of acute tolerance.

There are several studies in the literature that provide evidence of acute tolerance to some of the effects of nitrous oxide. For example, in mice, the loss of righting reflex was greater 5–6 min after inhalation onset of anesthetizing concentrations of nitrous oxide than 60–64 min after the onset of inhalation (11,43). In humans, at nitrous oxide concentrations of 60–80%, the analgesic effects of nitrous oxide were significantly greater between 2–120 min after the beginning of an inhalation period, than 150–180 min after the inhalation onset (37). In the same study, two of eight subjects who initially lost consciousness due to the high concentrations of nitrous oxide, regained consciousness between 70 and 100 min after inhalation onset, indicating the possibility of acute tolerance to the anesthetic effects of nitrous oxide. In another study, some subjects developed acute tolerance to the analgesic effects of nitrous oxide during a 46-min inhalation. However, the drug's effects were not consistent among subjects and there existed much intersubject variability in the development of acute tolerance (35). In still another human study, acute tolerance to the analgesic effects of 33 and 50% nitrous oxide was obtained over a 45-min period when heat and pressure were used as nociceptive stimuli (46). Another study established that human electroencephalographic response to continuous administration of nitrous oxide was altered as a function of time. While an initial administration of nitrous oxide produced a

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three-stage EEG pattern, a second administration bypassed stage one or stages one and two. The authors concluded that the electroencephalographic changes were in response to an alteration in CNS reactivity to nitrous oxide, indicative of acute drug tolerance (2). However, in two other human studies, acute tolerance did not appear to develop to the psychomotor impairing effects of 20 and 30% nitrous oxide during an inhalation interval of 40 min (21,28). It is certainly conceivable, though, that with a longer duration of inhalation than 40 min, acute tolerance may have developed.

No studies to date have determined whether acute tolerance develops to the subjective effects of nitrous oxide. Further, longer durations of nitrous oxide exposure may lessen the cognitive and psychomotor impairing effects of nitrous oxide. We believe there is clinical relevance to this study in that it would be important for medical professionals to know if patients receiving subanesthetic concentrations of nitrous oxide for extended duration conscious sedation procedures (e.g., dental work) are experiencing the same degree of psychotropic effects (e.g., sedation) at the end of the procedure as at the beginning. We recruited 11 subjects, exposed each to a range of nitrous oxide concentrations, and assessed multiple dependent measures including mood, cognition, and psychomotor performance. We sought to determine the presence and degree to which acute tolerance develops to these different measures of nitrous oxide effects, and if the occurrence of acute tolerance in humans is dose-dependent.

METHOD

Subjects

This study was approved by the local Institutional Review Board. Written informed consent from each subject was obtained prior to initiating the study. Subjects were told that the drug(s) to be used were commonly used in medical settings and may come from one or more of six classes delivered via a gaseous or aerosol form [i.e., sedative, stimulant, opiate, inhalational anesthetic (at subanesthetic doses), alcohol, or placebo (air that has no drug in it)]. Five females and six males [mean age (range): 22.8 (21–29)] participated. All subjects consumed alcohol on a regular basis [mean drinks consumed/week (range): 5.6 (0.4–12)], and eight subjects currently used marijuana [mean number of joints smoked/week (range): 1.1 (0.25–4)]. Seven subjects smoked tobacco cigarettes, but all smoked less than five per day [mean number of cigarettes smoked/day (range): 1.9 (0.1–4)]. Although the majority of subjects had some experience with marijuana, their lifetime use of this drug and other illicit drugs was generally light. Seven subjects had previous exposure to nitrous oxide; four had used it recreationally and three were exposed to it in medical procedures.

Prior to the first session, subjects attended a screening interview, at which time they completed the Symptoms Checklist-90 items (a questionnaire designed to assess psychiatric symptomatology) (8) and a health questionnaire to determine their psychiatric and medical status. A structured psychiatric screening interview was conducted by one of the research personnel. Candidates were excluded if they had any history of significant psychiatric disorders or substance use disorder (1), if they were taking any prescription medications (other than birth control pills), and if a significant history of neurologic, cardiac, pulmonary, hepatic, or renal disease, or any other medical contraindications, were found during a medical history and physical examination performed by a physician. Also, an electrocardiogram was performed to assure normal cardiac functioning. Prior to the first experimental session, urine drug toxicology screening was performed to determine that our subjects were not using PCP, amphetamines, cocaine, opioids, benzodiazepines, and barbiturates.

Subjects were instructed to refrain from using alcohol, illicit drugs, and over-the-counter medications for 24 h before and 12 h after the sessions. Subjects were told not to drive an automobile, operate heavy machinery, ride a bicycle, or cook with a stove until the day after the study, and were transported home after sessions. Payment for study participation was made following the last session, and a debriefing session was held after completion of the experiment.

Experimental Design

The experiment consisted of five sessions, each separated by at least 1 week. A randomized, crossover design was used. The study was double blinded in that the technician and subject were unaware of the drug or the dose being inhaled. The anesthesiologist administering the agent was aware of the drug, but had minimal verbal contact with the subject during the session. Effects of extended inhalation of 0, 10, 20, 30, and 40% nitrous oxide in oxygen were studied.

Experimental Sessions

Each session was approximately 190 min in duration and took place in the morning or in the afternoon. Generally subjects attended sessions at the same time of day throughout the experiment. Subjects had been instructed not to eat food or drink colored liquids (i.e., coffee and tea) for 4 h and not to drink any clear liquids (including water) for 2 h prior to sessions. Female subjects had to have a negative urine pregnancy test once per week while participating in the study. Subjects were given a breath alcohol test prior to beginning the experiment and were excluded from the upcoming session if any alcohol was detected (no alcohol was detected in any of the subjects). Subjects were seated in a chair during the entire testing period. Nitrous oxide and oxygen were delivered via a semiclosed circuit from an anesthetic machine (Narkomed, Draeger, Inc.; Telford, PA). Subjects inhaled through a clear anesthesia facial mask, that was affixed to the subject's face via attached rubber straps that went around the head. Oil of peppermint was placed in the circuit in an attempt to mask any differential odors between the drug and no-drug conditions. Noninvasive assessments of heart rate, electrocardiogram, peripheral oxygen saturation, and blood pressure were initiated at the beginning of the session and continuously monitored during the baseline and inhalation periods of the session.

Each session consisted of three periods: baseline (BL), inhalation, and recovery. During BL, subjects completed the entire battery of mood forms and tests, while inhaling oxygen through the mask. Subjects were told at this time that the air they were breathing did not contain drug in it. Upon completion of baseline testing, the anesthesiologist adjusted the anesthesia machine to deliver the appropriate concentration of nitrous oxide in oxygen for that particular session. Total flow rate was held constant at 5 l/min. Subjects were told that for the following 120 min they would be inhaling air that may or may not have a drug in it. The anesthesiologist stayed in the immediate vicinity for the 120 min during which subjects were inhaling placebo or nitrous oxide. At the end of the 120-min inhalation period, the mask was removed by the anesthesiologist and the 60-min recovery period commenced. At the prescribed intervals, mood, cognition, and psychomotor performance were assessed. When no tests were scheduled, subjects

were free to engage in sedentary recreational activities such as reading, listening to the radio or to cassette tapes, and watching TV. Studying was not permitted for two reasons: 1) nitrous oxide can cause confusion and amnesia, and subjects' affective responses to the drug might have been negatively affected by these two drug effects interfering with studying; and 2) we wanted to limit subjects' activities to one type of activity, rather than allowing subjects to study on some sessions and recreate on others (i.e., the two different activities could conceivably induce different mood effects).

Dependent Measures

Measures collected in this study consisted of self-reported subjective effects, cognitive and psychomotor performance, and physiological effects. The whole battery of testing took approximately 8–10 min to complete and order of the tests remained invariant throughout the experiment. At some time points in each session, only a subset of tests was administered from the battery, which took about 2 min to complete. All subjects had received practice on the psychomotor tests prior to the first experimental session.

Subjective effects. To assess subjective effects, we used three questionnaires. The Drug Effects/Liking questionnaire (locally developed) consisted of two items and assessed the extent to which subjects currently felt a drug effect, on a scale of 1 to 5 (1 = "I feel no effect from it at all"; 5 = "I feel a very strong effect"), and assessed the extent to which subjects liked the drug effect, on a 100-mm line (0 = dislike a lot; 50 = neutral; 100 = like a lot). The Drug Effects/Liking questionnaire was filled out at BL, 15, 40, 60, 85, and 105 min after initiation of the inhalation, and 5, 30, and 60 min after the inhalation had ceased.

The Visual Analogue Scale (VAS) consisted of 20 100-mm lines, each labeled with one of the following adjectives (locally selected): "stimulated," "high ('drug' high)," "anxious," "sedated," "dizzy," "tingling," "confused," "drunk," "elated (very happy)," "nauseous," "coasting or spaced out," "carefree," "down (depressed)," "having pleasant thoughts," "having unpleasant thoughts," "having pleasant bodily sensations," "having unpleasant bodily sensations," "in control of body," "in control of thoughts," "difficulty concentrating," and "hungry." Subjects were instructed to place a mark on each line indicating how they felt at the moment, ranging from "not at all" to "extremely". The VAS was filled out at BL, 15, 40, 60, 85, and 105 min after initiation of the inhalation, and 5, 30, and 60 min after the inhalation had ceased.

A computerized version of the short-form Addiction Research Center Inventory (ARCI), a true-false questionnaire (49 items) designed to differentiate among different classes of psychoactive drugs, was used (25). The 49 items yielded scores for five different scales: PCAG (Pentobarbital-Chlorpromazine-Alcohol group), a measure of sedation; BG (Benzedrine Group) and A (Amphetamine), measures of stimulant effects; LSD (Lysergic Acid Diethylamide), a measure of somatic and dysphoric effects; and MBG (Morphine-Benzedrine Group), a measure of euphoria. The ARCI was completed at BL, 15, 60, and 105 min after initiation of the inhalation, and 5, 30, and 60 min after the inhalation had ceased.

Cognitive/psychomotor functioning. To assess cognitive/psychomotor functioning, we used four tests. We used the backward digit span test as a general measure of cognitive functioning. The backward digit span test (45) is a computerized memory test, and presents strings of digits to subjects, which they are to reproduce in backwards order; successful completion of one trial initiates a succeeding trial in which the string of digits is incremented by one. The dependent measure is the number of trials successfully completed. The backward digit span test was completed at BL, 15, 60, and 105 min after initiation of the inhalation, and 5, 30, and 60 min after the inhalation had ceased.

Subjects completed two computerized psychomotor tests, auditory reaction time (29) and eye-hand coordination (29), and one paper-and-pencil psychomotor test, the Digit Symbol Substitution Test (DSST) (45). To measure auditory reaction time, the average time to depress a spacebar in response to 10 separate tones presented over 60 s at random intervals was measured. To measure eye-hand coordination skills, the subject traced a randomly moving target on the computer screen with a small cross controlled by a computer mouse for 1 min. Seconds outside of a 1-cm circle surrounding the randomly moving circle were measured. In the paper-and-pencil DSST, subjects for 1 min replaced a number with a corresponding symbol; the dependent measure was the number of symbols correctly drawn by the subject. Different forms of the test (i.e., different symbol-number codes) were used each time it was presented to the subject. The auditory reaction time and eye-hand coordination tests were administered at BL, 15, 60, and 105 min after initiation of the inhalation, and 5, 30, and 60 min postinhalation while the DSST was administered at BL, at 15, 40, 60, 85, and 105 min after initiation of the inhalation, and 5, 30, and 60 min postinhalation.

Physiological measures. Noninvasive assessments of heart rate, electrocardiogram, peripheral oxygen saturation, and blood pressure were initiated at the beginning of the session and recorded at BL, and 15, 60, and 105 min after initiation of the inhalation.

Data Analysis

Subjective and psychomotor effects data were analyzed using repeated measures analysis of variance (ANOVA). The

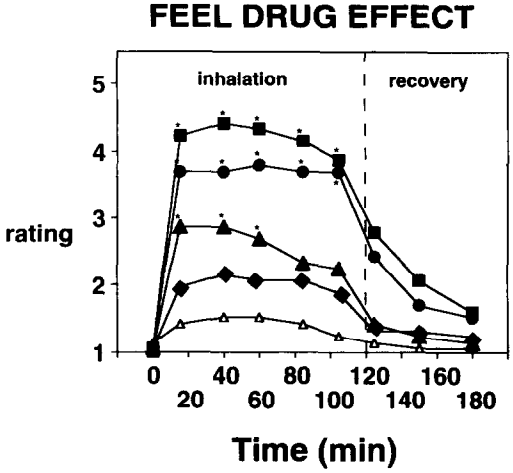

FIG. 1. Effects of 0% (open triangle), 10% (closed diamond), 20% (closed triangle), 30% (closed circle), and 40% (closed square) nitrous oxide inhalation on feel drug effect ratings as a function of time. Time point 0 refers to measures taken at baseline, and dashed vertical line separates the inhalation period of the session (2 h) from the recovery period (1 h). Ratings ranged from 1 = I feel no effect at all, to 5 = I feel a very strong effect. Asterisks represent significant differences from the 0% nitrous oxide (placebo-oxygen) condition.

TABLE 1
SELECTED SUBJECTIVE EFFECTS RATINGS

Minutes	0	15	60	105
Carefree (VAS)				
0% N_2O	9.6	13.8	15.2	15.5
10% N_2O	8.5	11.5	9.9	7.6
20% N_2O	8.6	14.2	15.2	12.4
30% N_2O	4.4	49.8*	30.8	38.6
40% N_2O	11	54.8*	52.8*	48.4*
Coasting or spaced out (VAS)				
0% N_2O	9.5	9.9	10.6	10.4
10% N_2O	3.8	15.1	12.2	8.4
20% N_2O	2.3	20.9	26.8	13.3
30% N_2O	2.8	46	45.2*	49.4*
40% N_2O	7.3	56.4*	63.8*	54.2*
Stimulated (VAS)				
0% N_2O	3	8.1	9.6	8
10% N_2O	3.8	11.9	6.1	4.6
20% N_2O	10.8	21.7	20.4	17.2
30% N_2O	13.1	42.6*	40.2*	38.9*
40% N_2O	6.9	40.8*	44.1*	37.5*
Having pleasant bodily sensations (VAS)				
0% N_2O	16.6	17.2	18.6	16.9
10% N_2O	16.5	20.4	17	18.2
20% N_2O	22.4	24.4	19.8	15.4
30% N_2O	15.7	42*	49.5*	43.6*
40% N_2O	16.7	55.6*	52.8*	50.7*
High (VAS)				
0% N_2O	2.3	8.4	8.9	9.3
10% N_2O	1.9	17.7	12.5	7.8
20% N_2O	1.2	29	34.6*	25.9
30% N_2O	2.4	61*	60.6*	54.2*
40% N_2O	1.7	70.7*	72.8*	64.1*
Liking (drug effects/liking questionnaire)				
0% N_2O	47.8	50.5	51.3	52.2
10% N_2O	48.5	49.6	47.8	49.4
20% N_2O	48.5	56.1	50.7	47.0
30% N_2O	48.6	66.5*	64.9	53.8
40% N_2O	46.5	79.6*	70.0*	62.0
LSD (ARCI)				
0% N_2O	3.3	2.6	2.9	3.0
10% N_2O	2.8	4.0	4.2	4.1
20% N_2O	2.9	3.3	3.8	3.8
30% N_2O	2.9	5.4*	6.2*	5.2*
40% N_2O	2.7	5.7*	5.6*	5.0*

*Represents significant difference when compared with same time point in the placebo-oxygen condition.

factors considered were nitrous oxide (five levels) and time (seven to nine levels). An effect was considered significant if $p < 0.05$. Huynh-Feldt adjustments of within-factors degrees of freedom were used to protect against violations of symmetry assumptions. Tukey post hoc comparison tests were employed when appropriate. Evidence of acute tolerance was gauged by determining whether any of the intrainhalation measures past the 15-min time point was significantly attenuated relative to the first intra-inhalation assessment time point (15 min).

RESULTS

Subjective Measures

Nitrous oxide significantly increased (dose and/or dose × time effects) ratings of feel drug effect ($p < 0.01$) in a concen-

tration-related manner (see Fig. 1). There was no evidence of a lessening of drug effect during the 120-min inhalation period. Subjects reported liking the effects of the drug ($p < 0.01$). Tukey post hoc testing revealed that liking ratings were significantly higher during inhalation of 30 and 40% nitrous oxide, relative to oxygen placebo. Nitrous oxide significantly increased, in a concentration-related manner, VAS ratings of carefree ($p < 0.01$), coasting ($p < 0.01$), confused ($p < 0.05$), difficulty concentrating ($p < 0.05$), dizzy ($p < 0.01$), drunk ($p < 0.01$), high ($p < 0.01$), nauseous ($p < 0.05$), sedated ($p < 0.01$), stimulated ($p < 0.01$), and tingling ($p < 0.01$), and significantly decreased ratings of in control of body ($p < 0.05$), and in control of thoughts ($p < 0.05$). Again though, there was no evidence of acute tolerance on any of these VAS measures during nitrous oxide inhalation. On the ARCI, nitrous oxide increased scores in a concentration-related manner on three of the five scales: PCAG ($p < 0.05$), MBG ($p < 0.05$), and LSD ($p < 0.05$). There was no evidence of acute tolerance on these measures. Table 1 lists several mean subjective effects ratings for the BL, 15-, 60-, and 105-min time points of each of the five nitrous oxide concentrations as well as delineating at which concentrations there was statistical significance on a given measure (relative to placebo-oxygen at the same time point). As discussed above, the magnitude of changes in ratings or scores induced by nitrous oxide were, in general, concentration dependent, with little change in ratings or scores across the 120-min inhalation period. It is interesting to note, though, that liking ratings were significantly lower during the latter part of the inhalation period. Tukey post hoc analysis of the 15-min time point compared with the 85- and 105-min time points during the inhalation revealed a significant decrease in drug liking ratings while subjects were inhaling 40% nitrous oxide. However, there was substantial intersubject variability regarding patterning of liking ratings during the 120-min period when 40% nitrous oxide was inhaled. Four subjects reported consistently liking the drug, two subjects remained neutral, two subjects reported a biphasic effect (first reporting liking, then reporting disliking as the session progressed), and three subjects reported a diminution across time (i.e., first reporting liking, then reporting neutral ratings).

Cognitive and Psychomotor Measures

Nitrous oxide impaired cognitive and psychomotor performance. Table 2 lists mean cognitive/psychomotor scores for the BL, 15-, 60-, and 105-min time points of each of the five nitrous oxide concentrations as well as delineating at which concentrations there was statistical significance on a given measure (relative to placebo-oxygen at the same time point). Nitrous oxide, at one or both of the higher concentrations, significantly impaired auditory reaction time ($p < 0.05$), eye-hand coordination (subjects spent more time outside of 1-cm circle) ($p < 0.05$), and number of symbols correctly completed on the DSST ($p < 0.01$) (see Fig. 2). Performance was impaired on the backward digit span test during nitrous oxide

TABLE 2

PSYCHOMOTOR/COGNITIVE PERFORMANCE SCORES

Minutes	0	15	60	105
Backward digit span (number of digits recalled)				
0% N_2O	6.0	6.5	6.2	6.1
10% N_2O	5.2	4.9	5.9	5.0
20% N_2O	5.4	5.6	5.5	4.5
30% N_2O	5.4	4.4	4.9	4.4
40% N_2O	6.0	3.6*	4.6	3.6
Auditory reaction time (s)				
0% N_2O	0.32	0.27	0.27	0.27
10% N_2O	0.28	0.27	0.27	0.29
20% N_2O	0.29	0.30	0.31	0.31
30% N_2O	0.28	0.30	0.34*	0.33*
40% N_2O	0.28	0.32	0.31	0.33*
Eye-hand coordination (seconds outside circle)				
0% N_2O	8.0	7.3	8.4	6.9
10% N_2O	7.1	6.7	9.5	6.2
20% N_2O	7.0	10.2	14.2	15.2
30% N_2O	6.3	10.6	15.9	12.2
40% N_2O	6.8	16.9	18.9*	19.2*
DSST (number of symbols correctly drawn)				
0% N_2O	52	52.5	53.7	51.9
10% N_2O	53.2	51	52.4	52.4
20% N_2O	55.1	52	49.9	52.1
30% N_2O	51.8	46.3	45*	47.9
40% N_2O	52.9	42.3*	41.4*	42.8*

*Represents significant difference when compared with same time point in the placebo-oxygen condition.

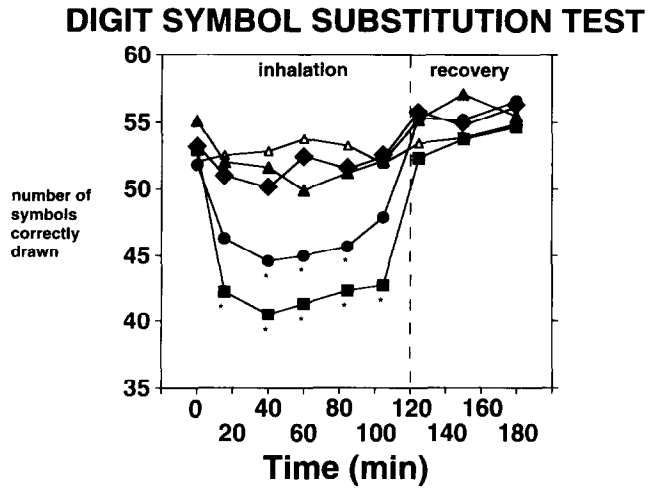

FIG. 2. Effects of 0% (open triangle), 10% (closed diamond), 20% (closed triangle), 30% (closed circle), and 40% (closed square) nitrous oxide inhalation on number of symbols correctly drawn on the DSST as a function of time. Time point 0 refers to measures taken at baseline, and dashed vertical line separates the inhalation period of the session (2 h) from the recovery period (1 h). Asterisks represent significant differences from the 0% nitrous oxide (placebo-oxygen) condition.

inhalation but results were statistically insignificant ($p = 0.09$). Generally, the magnitude of cognitive and psychomotor impairment was concentration dependent. The recovery of psychomotor/cognitive function was rapid, with performance returning to near baseline levels 5 min into the recovery period for most of the measures. As Fig. 2 exemplifies, and Table 2 shows, there was no evidence of acute tolerance to either the cognitive or the psychomotor impairing effects of nitrous oxide.

Physiological Measures

Statistically significant changes were not noted in any of the physiological parameters.

DISCUSSION

We found little evidence suggestive of acute tolerance to either the subjective or the cognitive/psychomotor impairing effects of nitrous oxide. Figures 1 and 2 show that subjective effects and psychomotor impairment remained relatively invariant throughout the inhalation part of the session.

Our findings, showing a lack of acute tolerance to the psychomotor impairing effects of nitrous oxide, are consistent with two previous studies (21,28) in which 20 and 30% nitrous oxide, over a 40-min inhalation, did not produce acute tolerance to the psychomotor-impairing effects of the drug. Two strengths of our study are that we assessed nitrous oxide effects for a longer period of time than other healthy volunteer studies that have assessed psychomotor performance, and that we assessed a wide range of subanesthetic concentrations of the study drug. Therefore, we feel we can state with a fair degree of confidence that acute tolerance does not develop to the cognitive and psychomotor impairing effects of a range of subanesthetic concentrations of nitrous oxide.

The significant decrease in drug liking ratings in the 40% nitrous oxide condition was not likely to be an example of acute tolerance. Only 3 of the 11 subjects showed a decreasing trend in liking of the drug effect as the inhalation period progressed; the other subjects showed a wide range of response patterns. This variability in drug liking has been obtained in previous studies from our laboratory (9,10,47) as well as other laboratories (36,44).

Our findings, taken in conjunction with other studies that have demonstrated acute tolerance to the anesthetic and analgesic effects of nitrous oxide (11,35,37,43,46), suggest that acute tolerance develops differentially to the myriad of effects of nitrous oxide. That is, acute tolerance appears to develop to the anesthetic and analgesic effects, but not to the subjective or psychomotor impairing effects of nitrous oxide. Taken in conjunction with the other studies, our findings also provide evidence that the different actions of nitrous oxide may be mediated by dissimilar neurochemical mechanisms. If the actions of nitrous oxide were mediated by one system, then given that acute tolerance develops to the anesthetic and analgesic effects of nitrous oxide, we should have found acute tolerance to the subjective and psychomotor effects of the study drug. Indeed, there is evidence for different neurochemical mechanisms mediating the different effects of nitrous oxide. A large number of studies, many using opioid antagonist challenges, have implicated the endogenous opioid system (EOS) in mediating the analgesic effects of nitrous oxide (3,4,19,27,33). In contrast, the anesthetic and subjective effects of the gas do not appear to be mediated by the EOS (14,41,48). While it is still not clear what neural mechanisms are involved in mediating the anesthetic effects of the gas, a recent study in humans suggested that the GABA-benzodiazepine-ionophore complex may be involved in mediating the subjective effects of nitrous oxide. In that study (49), flumazenil, a benzodiazepine receptor antagonist, attenuated the subjective rating of high during nitrous oxide inhalation. Further, a growing body of literature is also implicating the GABA-benzodiazepine-ionophore complex in mediating the anxiolytic effects of the drug (6,7,34).

Our findings of a lack of acute tolerance to the subjective, cognitive, and psychomotor effects of nitrous oxide, taken in conjunction with those studies showing acute tolerance to the anesthetic and analgesic effects of nitrous oxide, affirm the need to study multiple dependent measures in studying acute drug tolerance. Future studies should examine, within the same study, the effects of long-term administration of nitrous oxide on mood, cognition, psychomotor performance, and analgesia. We would predict a dissociation within the same study (i.e., acute tolerance to the analgesic effects of nitrous oxide without acute tolerance to the other effects of nitrous oxide). Analgesia was not assessed in this study to study the subjective and behavioral effects of nitrous oxide in the absence of stressful stimuli. There is a plethora of literature demonstrating the potency of different stressors in modulating the behavioral and physiological effects of different psychoactive drugs in both humans and infrahumans [e.g., (26,31, 32,39)]. We felt it was conceivable that the addition of a stressor, pain, in the present study, could have modulated the subjective and behavioral effects of nitrous oxide, thereby making any diminution, or lack of diminution, of these nitrous oxide effects across the inhalation part of the session difficult to interpret. Another interesting study to do in the future would be to examine whether, in humans, tolerance occurs to the analgesic, subjective, and behavioral effects of nitrous oxide after consecutive daily exposures to the same concentration of the gas. Certainly, tolerance to nitrous oxide after repeated exposure, as well as cross-tolerance between

the gas and drugs such as alcohol and morphine have been demonstrated in infrahumans (4,5,19,20,30,38,42). Finally, we believe our findings have relevance to health professionals (e.g., dentists, oral surgeons, anesthesiologists) who use nitrous oxide for conscious sedation procedures. It appears that, for the most part, the constellation of mood-altering effects of nitrous oxide would not change across the time course of a long procedure. Therefore, a patient sedated to a certain level at the beginning of a procedure would most likely be sedated at the same level one or two hours later into the procedure.

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