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Lack of Sex Differences to the Subjective Effects of Nitrous Oxide in Healthy Volunteers

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Abstract

Background—Although numerous studies have assessed subjective effects of nitrous oxide, few studies have analyzed for sex differences. Since sex differences have been reported in subjective effects of several drugs such as opioids, nicotine and alcohol, we sought to determine if sex modulates the subjective effects of the inhalant, nitrous oxide, in healthy volunteers.

Methods—Thirty-eight females and seventy-two males from nine studies that were conducted in our laboratory were included in this retrospective analysis. All experimental studies utilized randomized, placebo controlled, repeated measures designs in which subjects inhaled 30% nitrous oxide in oxygen and 100% oxygen (placebo). Dependent measures in this analysis were subjective effects measured at baseline and 15 min into the inhalation period.

Results—Nitrous oxide produced a number of subjective effects, including those that could be considered abuse liability-related (“elated,” “having pleasant thoughts,” drug liking), but sex did not modulate these effects.

Conclusions—Females and males showed similar subjective responses to 30% nitrous oxide. Future prospective studies might assess other concentrations, other measures (choice, analgesic response), and other inhaled general anesthetics to more comprehensively characterize the role of sex in response to inhalants.

Keywords

Nitrous Oxide; Subjective effects; Sex differences; Gender; Healthy volunteer

1. Introduction

Nitrous oxide (N₂O), or laughing gas, is a gaseous inhaled general anesthetic that has a long history of both use (Marshall and Longnecker, 1996) and abuse (Walker and Zacny, 2005). Numerous studies have examined the subjective effects of N₂O in male and female healthy volunteers, but few studies to the best of our knowledge have assessed for sex differences. We found only three studies in the extant literature that assessed for sex differences in which a placebo-controlled design was utilized - in two of the studies females reported greater effects from 40% N₂O than did males on some but not all subjective effects measures

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(Dohrn et al., 1992, 1993), and in the other study, it was stated that sex differences when inhaling 30% N₂O were rare (Block et al., 1990). These studies had small sample sizes (i.e., 6-8 males and 6-8 females exposed to N₂O), calling into question whether sex differences were adequately tested. In another study using a larger sample, a greater percentage of females (44.5%) than males (17.7%) reported the experience of inhaling N₂O (range: 40-80%) as unpleasant (Rosenberg, 1974). However, this study did not utilize a placebo control condition. Sex differences have been reported to the subjective effects of numerous drugs, including opioids (Comer et al., 2010; Zacny and Drum, 2010), cocaine (Sofuoglu et al., 1999; Lynch et al., 2008), nicotine (Perkins et al., 1999, 2009), delta-9-tetrahydrocannabinol (Haney, 2007), alcohol (Fillmore and Weafer, 2004), *d*-amphetamine (Van Sickel et al., 2007, 2010), and ecstasy (Liechti et al., 2001). In some of the studies the sex differences were quantitative in nature with females reporting either stronger (e.g., Liechti et al., 2001) or weaker (e.g., Haney, 2007; Lynch et al., 2008) effects from a drug than males, and in other studies the differences were qualitative in nature with males reporting more positive effects from a drug than females (e.g., Perkins et al., 2009; Comer et al., 2010). Given the fact that sex differences have been found with other psychoactive drugs, we thought it would be worthwhile to revisit the issue of whether the subjective effects of the inhalant, N₂O, were modulated by sex. We examined this in a retrospective fashion by pooling data from nine studies conducted in our laboratory. Although the studies differed in certain aspects (e.g., purpose, other doses, and in some cases other drugs studied) they had the following things in common: dose conditions included 100% oxygen and 30% N₂O in oxygen; the testing batteries included a visual analog scale to assess mood; and assessment time points included baseline and 15 min into the inhalation period (Zacny et al., 1994; Zacny et al., 1996a; Yajnik et al., 1996; Cho et al., 1997; Zacny et al., 1997; Zacny et al., 1998; Zacny et al., 1999). These commonalities allowed us to assess for potential sex differences in the subjective effects of N₂O in a relatively large sample of volunteers.

2. Methods

2.1. Subjects

In order to be eligible for the studies, volunteers needed to 1) be within the age range of 21-39 years; 2) have a high school education or an equivalent; 3) be verbally fluent in English; 4) be within 20% of their ideal body weight as determined by standard height-weight tables (Metropolitan Life Insurance actuarial tables); and 5) have at least some current level of recreational drug usage. Usage for most studies was defined as consumption of 3 or more alcoholic drinks within a month or some [1 joint/week] but not daily use of marijuana. One study examined N₂O effects in light and moderate drinkers, and the alcohol consumption use criteria differed from that stated above, but those different criteria were the same for males and females (Cho et al., 1997). All subjects had to be in good health, which was ascertained by passing a physical examination and having a normal resting EKG. Subjects were excluded for having a history of psychiatric or substance use disorders as determined from a structured interview using DSM-III-R or DSM-IV diagnostic criteria (American Psychiatric Association, 1987, 2000) and smoking more than five cigarettes daily. All studies were approved by the Institutional Review Board, and written informed consent from each subject was obtained prior to initiating a study.

2.2. Experimental design

All experimental studies utilized randomized, placebo-controlled, repeated measures designs. In two studies there was a grouping factor (i.e., mixed model design) (Cho et al., 1997; Zacny et al., 1997). All studies except one (Zacny et al., 1997) used a double blind design. In the Zacny et al. (1997) study one group was blinded and another group was informed as to what they were inhaling – only the former group is included in the

retrospective analysis. Four studies (Zacny et al., 1994; Zacny et al., 1998; Zacny et al., 1999) involved administering another drug (naloxone or alcohol) in conjunction with N₂O, and in these studies we only used data in which the placebo condition was used for the other drug.

2.3. Procedures

The studies took place in a laboratory within a hospital. Sessions were typically conducted in the afternoon hours. Prior to each session, breath alcohol testing and pregnancy screening (for females) were conducted. In some studies urine toxicology screening was conducted before every session and others on only some of the sessions (in those studies, subjects were not aware of when they would be tested). Negative test results were required for sessions to proceed.

During sessions, subjects were seated in a reclining chair. Non-invasive assessment of heart rate, peripheral oxygen saturation, and blood pressure was initiated at the beginning of a session, and was monitored throughout sessions. Prior to each inhalation period (i.e., baseline period), a clear anesthesia facial mask was affixed to the subject's face via attached rubber straps that went around the head. During baseline testing, subjects were told that the air they were breathing was drug-free air (i.e., 100% oxygen). Upon completion of baseline testing the anesthetist administered either 30% N₂O in oxygen or 100% oxygen for a certain duration of time (ranging from 30-120 min), and subjects were told that the air they were now inhaling may or may not contain a drug. N₂O and 100% oxygen were delivered via a semi-closed circuit from an anesthesia machine at a flow rate of 5 L/min.

Across the nine studies, N₂O and 100% oxygen (placebo) were administered either within the same session or in separate sessions. The agents could be administered within the same session because the residual effects of N₂O, once it is no longer being administered, are extremely short (Beckman et al., 2005). In the studies in which the agents were inhaled in the same session (Zacny et al., 1996b; Cho et al., 1997; Zacny et al., 1997), a period of 60 minutes in which the mask was removed separated the two inhalation periods. Order of N₂O presentation was either randomized or counterbalanced with other drug conditions in each of the studies. All inhalation periods commenced with baseline testing. Subjective effects testing with the Visual Analog Scale (VAS) and the Drug Effect/Liking questionnaire (DE/L) occurred at baseline and 15 minutes into the inhalation period. Depending on the study, the VAS and DE/L may have been administered more than once during an inhalation period but for our purposes we focused on the 15-min time point. Using an inhalation simulation software program that estimates end-tidal volumes of different inhaled anesthetic agents (i.e., a proxy for concentrations in the CNS) [Gas Man® San Ramon, CA], it was estimated that volunteers were exhaling 25% N₂O when they were undergoing the battery of testing 15 min intra-inhalation.

2.4. Dependent measures

2.4.1. Subjective Effects—The Visual Analog Scale (VAS) was locally developed and depending on the study consisted of twenty to twenty-six 100-mm lines, each labeled with an adjective or phrase. 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 nine studies in this retrospective analysis had 10 adjectives or phrases in common, and eight of the nine had an additional 4 adjectives or phrases in common. A total of 14 items were therefore subjected to statistical analysis with four of the analyses having slightly smaller sample sizes. The Drug Effects/Liking questionnaire (DE/L) 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 on a 100-mm lines assessed the extent to which subjects currently liked the drug effect (0=dislike a lot; 50=neutral; 100=like a lot).

2.4.2. Demographic Measures—Measures included age, body weight, body mass index, race, ethnicity, current drug use, and lifetime nonmedical use of drugs.

Data analysis: The modulating effects of sex on subjective effects of N₂O were analyzed using a mixed model analysis of variance (ANOVA) with the factors Sex, N₂O Concentration (0 and 30%), and Time (0 min [baseline] and 15 min into the inhalation period). Huynh-Feldt adjustments of within-factors degrees of freedom were used to protect against violations of sphericity. When results of ANOVA were significant ($p \leq 0.05$), Tukey post hoc comparison tests were used to identify which differences were statistically significant. T-tests and Fishers Exact Test were used in analyzing demographic measures.

3. Results

One hundred ten subjects were included in the analyses (38 females, 72 males). Table 1 presents demographics of subjects. Males on average weighed more and had higher BMIs than females, but weight does not affect the pharmacokinetics or pharmacodynamics of N₂O (Stanski, 1990). Males reported consuming a greater number of alcoholic and caffeinated beverages per week than females. A greater proportion of females than males reported smoking. Self reported prevalence of lifetime recreational use of marijuana, hallucinogens, stimulants, inhalants, sedatives, ecstasy and opioids did not differ between males and females.

Table 2 presents subjective effects measures significantly affected by N₂O relative to placebo (i.e., significant Drug $\times$ Time interactions) separately for males and females. Sex did not modulate the subjective effects of N₂O (i.e., no Sex $\times$ Drug or Sex $\times$ Drug $\times$ Time interactions). Post hoc testing of the Drug $\times$ Time interactions (data not shown) revealed that in all cases, ratings at the 15 min time point in the N₂O condition differed significantly from ratings at baseline and from ratings at the 15 min time point in the placebo condition. Not shown in Table 2 are those subjective effects not affected by N₂O - VAS ratings of “down,” “having unpleasant bodily sensations,” “having unpleasant thoughts,” and “nauseous.”

4. Discussion

There have been numerous studies conducted over the past half century on the subjective effects of N₂O (Walker and Zacny, 2005), but we could find very few studies that examined whether the effects differed as a function of sex. This is somewhat surprising given that other psychoactive drugs' effects have been shown to differ between males and females. Our retrospective analysis indicates that there does not appear to be differences in how male and female healthy volunteers report feeling from the effects of 30% N₂O. Notably both males and females reported an increase in positive effects (e.g., “elated [very happy],” “having pleasant bodily sensations,” drug liking) and for the most part an absence of dysphoric effects (i.e., no increases in ratings of “down,” “having unpleasant bodily sensations”). These results would suggest that the liability of abuse of N₂O in males and females is similar. The actual prevalence of recreational use of N₂O as a function of sex is not known. The United States annual National Survey on Drug Use and Health (NSDUH) reports on lifetime prevalence rates of use of specific inhalants, including N₂O, and in 2008 4.5% of respondents aged 12 years and older reported use of it for “kicks” or “to get high,” but how many of them were males versus females was not reported (NSDUH, 2009).

The present study had several limitations. This was a retrospective analysis which involved pooling data from studies that differed on a number of aspects (e.g., length of sessions, drugs tested, subject inclusion criteria). The extent to which these differences impacted on the overall results of the retrospective analysis is not known. We limited our analysis to one concentration of inhaled N₂O (30%), measured at one time point. There may be sex differences at higher doses of N₂O as was reported in the Rosenberg (1974) study that tested doses of 40-80% N₂O. There were substantially more males than females in the analysis. We did not document where females were at in their menstrual cycle during studies, and some female subjects were on hormonal contraceptives while others were not. Given such limitations, caution must be used when drawing conclusions from this study. We believe it would be worthwhile in a prospective study or studies to assess other doses, other measures (choice, analgesic response), and other inhaled general anesthetics (e.g., volatile anesthetics such as sevoflurane) to more comprehensively characterize the role of sex in response to inhalants.

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Table 1

Participant demographics.

	Females	Males
Group size (n)	38	72
Age (years; M±SD)	24.6 (4.6)	25.5 (4.2)
Weight (kg; M±SD)	62.3 (7.3)*	77.0 (9.9)
BMI (M±SD)	21.6 (2.1)*	23.5 (2.2)
Race		
Caucasian	29 (76.3%)	61 (84.7%)
African American	1 (2.6%)	2 (2.8%)
Asian	3 (7.9%)	2 (2.8%)
American Indian/Alaskan Native	0 (0%)	1 (1.4%)
Multiracial	1 (2.6%)	1 (1.4%)
Unknown	4 (10.5%)	5 (6.9%)
Current drug use (past 30 days)		
Alcohol (drinks/week; M±SD)	3.2 (3.7)*	4.9 (4.1)
Caffeine (drinks/week; M±SD)	8.6 (5.7)*	12.2 (12.0)
Smokers (n; all <5 cigarettes/day)	16 (42.1%)*	14 (19.4%)
Marijuana (n)	11 (28.9%)	24 (33.3%)
Lifetime recreational drug use (n)		
Marijuana		
Never used	6 (15.8%)	15 (20.1%)
Used <10 times	7 (18.4%)	19 (26.4%)
Used 10-50 times	17 (44.7%)	23 (31.9%)
Used >50 times	8 (21.1%)	15 (20.1%)
Hallucinogens	16 (42.1%)	32 (44.4%)
Stimulants	10 (26.3%)	19 (26.4%)
Inhalants	11 (28.9%)	22 (30.6%)
Sedatives	6 (15.8%)	7 (9.7%)
Ecstasy	5 (13.2%)	6 (8.3%)
Opioids	3 (7.9%)	8 (11.1%)

* significant difference from males

Table 2

Mean values of subjective effects measures significantly affected by nitrous oxide (N₂O) relative to placebo (PLC). Data from baseline (BL) and the 15 min intra-inhalation time points are presented separately for females and males.

	Females				Males			
	PLC (100% O ₂)		30% N ₂ O		PLC (100% O ₂)		30% N ₂ O	
	BL	15 min	BL	15 min	BL	15 min	BL	15 min
Subjective Effects								
VAS								
Coasting ('spaced out') ^a	4.7	7.8	3.7	32.3	2.1	2.0	1.4	24.2
Confused ^a	1.7	2.1	1.9	12.4	0.9	1.0	1.5	10.6
Drunk ^a	1.5	1.9	2.1	17.7	1.2	1.1	1.3	18.1
Elated (very happy) ^a	4.5	4.0	5.0	24.0	8.0	6.3	7.8	22.7
Having pleasant bodily sensations ^b	8.8	9.1	11.7	32.7	10.9	11.0	10.8	33.7
Having pleasant thoughts ^b	19.5	15.3	20.9	36.5	19.6	18.3	20.8	36.3
High ('drug' high) ^a	1.6	3.5	1.8	43.1	1.2	1.7	1.3	42.4
Sedated (calm, tranquil) ^a	9.6	10.9	9.1	24.5	8.7	8.2	9.1	20.5
Stimulated (energetic) ^a	6.8	5.8	7.8	28.1	11.1	8.5	11.3	30.1
Tingling ^a	1.4	2.5	2.0	39.8	1.1	1.5	1.1	31.4
DE/L								
Drug effect ^a	1.1	1.5	1.0	3.6	1.1	1.5	1.1	3.3
Liking ^a	48.7	51.9	49.0	61.4	49.0	48.2	48.7	62.3

^aUsed in 9 studies; 72M, 38F (37F for "Coasting (spaced out)"; 71M for "Liking")

^bUsed in 8 studies; 63M, 35F