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Nitrous oxide analgesia in humans: Acute and chronic tolerance

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Abstract

Electrical tooth stimulation was used to investigate whether humans develop tolerance to nitrous oxide (N₂O) analgesia within a single administration as well as over repeated administrations. In a double-blind cross-over experiment, 77 subjects received a 40-minute administration of 38% N₂O at one session and placebo gas at the other. The sessions were separated by 1 week and the order of gas administration was counterbalanced. Acute analgesic tolerance developed for pain threshold but not for detection threshold. There was no evidence of a hyperalgesic rebound effect following cessation of the N₂O administration. In a second double-blind experiment, 64 subjects received both 30-min of placebo gas and 30-min of 35% N₂O, separated by a 35-min gas wash-out period, during each of 5 sessions. Sensory thresholds were assessed prior to drug or placebo administration (baseline) and between 7-12 min and 25-30 min of gas administration. A control group of 16 subjects received only placebo gas at these 5 sessions. During a sixth session, the experimental procedures were similar to the previous sessions except that the control group received N₂O for the first time and the experimental group was sub-divided to test for conditioned drug effects. For both detection and pain threshold measures, acute tolerance developed during the initial N₂O exposure and chronic tolerance developed over repeated administrations. Although chronic tolerance developed, a test for Pavlovian drug conditioning found no evidence of conditioned effects on sensory thresholds. In conclusion, acute and chronic tolerance develop to N₂O's analgesic effects in humans.

Keywords

Pavlovian drug conditioning; pain; individual differences; electrical tooth stimulation; rebound; pain threshold

1. Introduction

Chronic tolerance typically is described as a reduction in a drug's effect when a constant drug dose is repeatedly administered. Acute tolerance occurs when a drug's effectiveness diminishes during the course of a single administration (Kalant et al., 1971). Acute tolerance is best studied during an initial drug administration because the variable being measured is less confounded by the chronic tolerance that develops with repeated exposures (Ramsay and Woods, 1997).

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Nitrous oxide (N₂O) is a pharmacologically active gas with a modest analgesic effect at subanesthetic concentrations (Finck, 1985; Maze and Fujinaga, 2000; Quock and Vaughn, 1995). Human studies of N₂O analgesia led to the observation that acute tolerance may develop to this effect. For example, Persson (1951) noted that the analgesic action of 30% N₂O sometimes dissipated during its administration, and he suggested this might result from either the person's "accommodation" [sic] (p. 85) to the drug or that "another factor prevails which counteracts the hypalgesic action of nitrous oxide" (p. 56). In a human study using 33% N₂O, Whitwam and colleagues (1976) suggested that the nervous system adapts to the analgesia that results from a constant concentration of nitrous oxide. Large individual differences in acute tolerance to the analgesia caused by 35-40% N₂O were also noted by Ramsay and colleagues (1992). Using cold-pressor pain, acute tolerance was not observed during a 40-minute administration of up to 40% N₂O (Pirec et al., 1995) but was found during a 120-min administration of 30-40% N₂O (Zacny et al., 1996). Animal research suggests that tolerance develops to N₂O's antinociceptive effects (Berkowitz et al., 1977; Berkowitz et al., 1979; Ruppreht et al., 1984), but this finding is not universal (Shingu et al., 1985) and may depend on rat strain (Fender et al., 2000).

Human data suggest that acute tolerance develops to N₂O's hedonic effects (Zacny et al., 1996) but not to its subjective, cognitive, or psychomotor effects (Kortilla, et al., 1981; Moore, 1983; Yajnik et al., 1996; Zacny et al., 1996). Humans develop acute tolerance to anesthetic concentrations of N₂O (Ruppreht et al., 1985). Similarly, when N₂O is combined with other anesthetic drugs to cause anesthesia, tolerance develops to N₂O on an electroencephalographic measure (Avramov, et al., 1990). Animal research has documented tolerance development to N₂O using non-pain related measures (Dzolic et al., 1994; Ramsay et al., 1999; Smith et al., 1979a).

One human study (Haugen et al., 1959) examined the consequences of repeated N₂O exposure on pain thresholds as determined by electrical tooth stimulation. The analgesic effectiveness of 40% N₂O was reduced after four brief exposures such that the threshold values no longer differed from control levels. However, the N₂O exposures in that study were too brief to achieve a known or stable systemic concentration.

The present research investigated acute (Experiment 1) and chronic (Experiment 2) tolerance development to N₂O analgesia in humans. The chronic tolerance study was conducted within the context of a Pavlovian conditioning procedure to evaluate whether associative processes may play a role in chronic tolerance development to N₂O analgesia.

2. Methods

2.1. Subjects

Male and female subjects (age range 18-46) were recruited from posters placed on the University of Washington campus. Individuals with significant medical problems or females who were, or might be, pregnant were excluded. Because the gas was delivered via a nasal mask, all subjects had to be able to breathe freely through the nose at the time of the study. During the course of the research, having a moustache was added as an exclusion criterion because of the difficulty in creating a tight seal between the nasal mask and the upper lip. In addition, subjects were instructed not to take analgesic medications within 24 hours of any scheduled test session. Subjects received monetary compensation for their participation in this research. These studies were approved by the local Institutional Review Board.

2.2 Apparatus

2.2.1 Gas and Odor Delivery System—To administer the N₂O or placebo gas, a computer-controlled solenoid valve (Parker Hannifin, Fairfield, NJ) delivered either nitrogen (N₂) or N₂O to a commercially available N₂O and oxygen (O₂) two-gas blender (Bird, Palm Springs, CA). Subjects assigned to receive N₂O were administered 38% N₂O and 62% O₂ in Experiment 1 and 35% N₂O and 65% O₂ in Experiment 2; subjects assigned to receive placebo were administered 38% N₂ and 62% O₂ in Experiment 1 and 35% N₂ and 65% O₂ in Experiment 2. An infrared gas analyzer (Datex model #CD202; Helsinki, Finland) continuously measured N₂O, O₂, and carbon dioxide concentrations in gas sampled from the subject's nostril via a nasal cannula. The N₂O concentration was not displayed to the operator or the subject. A software program written in LabVIEW (National Instruments, Austin TX) controlled whether the N₂O or placebo gas was delivered and acquired the gas concentration data from the infrared gas analyzer. Gas flow rates were adjusted for each subject so that the gas delivery unit's reservoir bag always had sufficient gas to meet the subject's inspirational demand. Medical grade gasses were used.

To provide an odor cue in association with the gas being delivered, the gas stream going to the subject was diverted through either of two bubble-through respiratory gas humidifiers (Hudson Oxygen Therapy Sales Co., Temecula, CA) containing a mixture of water and a scented extract. In Experiment 1, one humidifier contained 330 ml of water and the other contained 330 ml of water mixed with 0.5 ml of almond extract (Schilling). In Experiment 2, one humidifier contained 330 ml of water mixed with 0.5 ml of almond extract and the other contained 330 ml of water mixed with 0.5 ml of anise extract (Crescent).

2.2.2 Auditory Cue Generator—In Experiment 2, distinct sounds were used as part of the stimulus configuration that was paired with the drug administration. The two audio signals were generated from computer-controlled digital-to-analog waveform tables. The signals were either a tone or clicks. The tone was a 455 Hz square wave signal. The clicks were from a 6.8 Hz spike signal created by a waveform table loaded with a 3% duty cycle square. The decibel level was equalized between the two sounds. The sounds were played in the testing room through a small audio amplifier-speaker system. The volume control was set by the operator to be at a comfortable level for the subject.

2.2.3 Electrical Tooth Stimulator: The electrical tooth stimulator was based on the computer-controlled, capacitive discharge device described by Brown and colleagues (1984, 1985). It uses a high voltage power supply to charge a known capacitor to a given voltage. Fast, high voltage relays are used to switch the capacitor from the charging circuit to the tooth stimulating electrodes, a process which discharges the capacitor through the tooth. The main reasons for selecting this type of tooth stimulator are safety and amenability to computer control. Safety is a major consideration in human tooth stimulation because the high resistance of enamel means that several hundred volts can be required to reach sensory threshold. The switched capacitor arrangement achieves almost perfect electrical isolation and limits the amount of energy that can be delivered should the electrodes accidentally contact low resistance tissue. The voltage to which the capacitor is charged is readily computer controlled by means of a digital-to-analog converter and a high voltage amplifier. The tooth stimulator also contains a circuit to monitor the tooth's impedance. This measure of impedance was used to identify an open circuit (e.g., the stimulating electrode out of contact with the tooth) or salivary contamination of the tooth resulting in a low impedance alternate path (e.g., effectively a short circuit) for the electrical stimulus.

The capacitances and voltages for which the stimulator is designed lead to a stimulus duration of about 1 millisecond (msec) when the impedance of the tooth being stimulated is

approximately 1 megaohm ($M\Omega$). Thus, it is straightforward to convert the stimuli expressed in units of charge (nanocoulombs, ncoul) to units of current (microamperes, μA) because ncoul per msec equals μA . Given a stimulus duration of approximately 1 msec, the numerical value of the stimulus amplitude is similar in both units (e.g., a discharge of 1 ncoul in 1 msec represents a mean current of 1 μA). The computer-controlled tooth stimulator was configured to deliver an electrical impulse that ranged in amplitude from zero ncoul to a maximum of 320 ncoul. The amount of charge per stimulus and the inter-stimulus interval were controlled using a software program written in LabVIEW.

2.3 Tooth Stimulation Procedure

Psychophysical pain assessment was performed using a stimulus-dependent method (Gracely et al., 1988) that allows the intensity of the stimulus to vary until a specific response is evoked, and that stimulus intensity is defined as the dependent variable. The primary dependent variables obtained from the tooth stimulation protocols were a subject's detection threshold and pain threshold. Detection threshold was calculated as the mean of the highest intensity non-detectable stimulation and that of the first detected sensation. Pain threshold was calculated as the mean of the highest perceived but non-painful stimulus and the first painful stimulus.

To prevent salivary contamination from degrading the electrical stimulus delivered to the tooth, a rubber dam (Quick Dam[®] by Vivadent Co., Schaan / Lichtenstein) was placed over the maxillary anterior teeth to isolate them from the oral cavity (Champion et al., 1991) and to provide a dry field for tooth stimulation and to form a tight seal against the lips, minimizing dilution of the nasally administered N_2O by mouth breathing. Electrical stimuli were delivered to a tooth via a 30-ga silver-plated copper wire which abutted the labial surface of the middle third of the tooth's crown by means of an acrylic splint containing a circular hole (2.5 mm diameter) filled with conductive gel. The electrode on the tooth served as the negative electrode and a positive electrode (a disposable infant ECG electrode) was attached to the cheek to complete the circuit.

All subjects were initially trained in the tooth stimulation procedure. Subjects held a response button and received a series of electrical impulses of gradually increasing charge. Subjects were instructed: 1) not to press the button unless they felt a tooth sensation; 2) to press the button once each time they felt a tooth sensation that was discernible but not painful; and 3) to press the button twice if a sensation was painful. During training, each ascending "staircase" pattern of stimulations began with a 5 ncoul stimulus, which is below a subject's detection threshold, and ended when a subject first reported feeling pain. The amount of charge increment (i.e., the step size) between successive electrical stimuli was typically 5 ncoul, but this could be adjusted for each subject to achieve approximately 5 to 9 perceptible but non-painful stimuli prior to the subject reporting a painful sensation. A constant inter-stimulus interval (2 sec) was used during training. Subjects were also taught that holding down the response button would stop the stimulation. Once subjects were trained using the simple ascending staircase method, a more complex pattern of "self-adjusting" staircases was employed so that subjects could not anticipate or predict the timing / intensity of the next stimulus. The interval between any two stimuli was randomly selected from a set of 9 possible intervals with a mean of 2 sec and a range of 1.6 to 2.4 sec.

2.4 Preliminary Exam and Training Sessions

Individuals interested in participating in these studies were scheduled for an initial exam where the study was described and informed consent was discussed. If consent was received, a subject's health history was obtained and several surveys were completed. A dental exam was conducted to ascertain the health and vitality of the maxillary anterior teeth and an acrylic splint

was fabricated to fit over the maxillary canines and incisors. A second session was then scheduled to train the subject in the electrical tooth stimulation procedure.

During training, each subject was asked to breathe room air through the nasal mask in order to more fully simulate the gas administration procedures that would take place during the test sessions. The subject reclined in the dental chair, the rubber dam was placed and stimulating electrodes were connected to two maxillary teeth (typically incisors and sometimes canines) using the acrylic splint. A simple ascending staircase was alternated between two teeth for a total of two ascending staircases per tooth. After a brief pause, the two teeth were again alternated until each received two 3-min episodes of the self-adjusting staircase. The training session ended with one ascending staircase per tooth. Based on the subjects who completed both experiments, the training session preceded the first experimental session by an average of 12.5 days (range = 6 - 62 days) in Experiment 1 and by an average of 13.7 days (range = 6 - 30 days) in Experiment 2.

2.5 Experimental Design and Procedures

2.5.1 Experiment 1: Acute Tolerance and Rebound Effects—The study design was a double-blind cross-over experiment where all subjects received a 40-minute administration of 38% N₂O at one session and a comparable administration of placebo gas at the other. The two test sessions were separated by an average of 10.3 days (range = 6 - 46 days) and the order of gas administration was balanced such that 39 of the subjects received N₂O on the first test session.

Both sessions began by connecting the subject to the tooth stimulator and administering 62% O₂ and 38% N₂. The same incisor was tested at both sessions. Each session began with 2 ascending staircases to re-acquaint the subject with the procedure. This was followed by 4-min of self-adjusting staircases that established the baseline detection and pain threshold values. After the baseline assessment was complete, 40-min of almond scented gas was administered which was either 38% N₂O or placebo depending on the session. A 4-min episode of self-adjusting staircases occurred from 7-11 min and from 36-40 min during the gas exposure. After the 40-min gas administration, all subjects received the almond scented placebo gas for an additional 24 minutes. This 24-min period was composed of alternating periods of 4-min of rest and 4-min of self-adjusting staircases.

2.5.2 Experiment 2: Chronic Tolerance and Associative Processes—The chronic tolerance study (Fig. 1) was a double-blind experiment where subjects in the experimental group (N = 64) received both a 30-min administration of 35% N₂O and a 30-min administration of placebo gas during each of the first five sessions. [The 38% N₂O concentration used in the first experiment was reduced slightly to 35% N₂O in an attempt to reduce subject drop-out.] The order of N₂O and placebo gas administrations within the five sessions was counterbalanced in such a way that two subjects were assigned randomly to each of the 32 possible orderings. The two gas administrations during each session were separated by a 35-minute administration of 35% N₂ and 65% O₂. At each of the five sessions, one of two distinct sets of cues (a cue consisted of a combination of light, sound, and odor) was paired consistently with placebo gas and the other cue with N₂O. The cue-gas pairings were randomly assigned to subjects and were counterbalanced with the gas administration orderings. A control group (N = 16) was treated similarly to the experimental group except that both gas administrations during a session were placebo gas. The five sessions took place in consecutive half-day blocks (i.e., morning and afternoon) from Wednesday through Friday morning. Each control group subject was randomly assigned to one of the two cue-gas pairings (8 subjects each).

Session 6 took place on Friday afternoon. During this session, the 64 subjects in the experimental group were randomly divided into 4 groups so that the first gas exposure of the

session would be either: 1) placebo gas and the cue paired with placebo, 2) placebo gas and the cue paired with N₂O, 3) N₂O and the cue paired with N₂O, or 4) N₂O and the cue paired with placebo. To facilitate further analysis, subjects received a second exposure to the same gas but the opposite cue. The 16 subjects in the control group were assigned to receive both N₂O and placebo gas as well as exposure to both cues. The orders of gas administration and cue presentation were counterbalanced. Only data from the experimental group's first gas exposure in Session 6 are presented.

The preliminary training session was modified slightly from the first experiment so that four, rather than two, maxillary anterior teeth were tested. The three teeth that appeared most reliable were selected for testing during the subsequent six experimental sessions. Of the three teeth selected, the most reliable one was stimulated during Sessions 1 and 6, the next most reliable tooth was tested during Sessions 2 and 4, and the remaining tooth was tested during Sessions 3 and 5. All sessions began by connecting the subject to the tooth stimulator and administering 65% O₂ and 35% N₂. During each session, there were two 30-min odorized gas administrations. Prior to each gas administration, a 4-min episode of self-adjusting staircases established the baseline detection and pain threshold values. During the odorized gas administration, two additional 5-min episodes of self-adjusting staircases were conducted between 7-12 and 25-30 min of the 30-min gas administration.

Two distinct sets of cues were used during the experiment, a blue light, a tone and almond odor or else a yellow light, a clicking sound and anise odor. The odors were delivered in a pulsatile fashion of 45 sec of odorized gas followed by 30 sec of non-odorized gas. Subjects in the experimental and control groups always had each cue presented concurrently with one of the two 30-min gas administrations during a session. For the first five sessions, each subject in the experimental group had a consistent pairing of one cue with placebo and the other cue with N₂O.

2.6 Data Analysis

The primary units of analysis were the maximum detection threshold and maximum pain threshold during each assessment period. The maximum threshold was selected as the summary measure because it is clearly understood as the largest stimulus considered painful during each period, it is unaffected by low outlying threshold values due to accidental button presses by subjects, and, unlike other statistics such as the mean, the maximum retains its interpretation in the presence of threshold values that are right-censored at the maximum intensity provided by the stimulator (320 ncoul).

2.6.1 Data Analysis in Experiment 1—The drug effect and acute tolerance development were assessed by within-subjects analyses that compared responses during the N₂O and placebo exposures using paired t-tests. The response variable used to assess the drug effect was the change in maximum sensory threshold from baseline to 7-11 minutes. The change from 7-11 minutes to 36-40 minutes was used to assess acute tolerance.

2.6.2 Data Analysis in Experiment 2—The experimental design allowed two different methods for assessing the initial drug effect and acute tolerance development during the initial exposure session: 1) a between-subjects analysis that compared the initial N₂O exposure in Session 1 for the experimental group with the average of the two placebo exposures in Session 1 for the control group using two-sample t-tests (using the Satterthwaite method (Armitage et al., 2002) for accommodating unequal variances), and 2) a within-subjects analysis of the experimental group that compared responses during the N₂O and placebo exposures in Session 1 using paired t-tests. The response variable used to assess the initial drug effect was the change in maximum sensory threshold from baseline to 7-12 minutes. The change from 7-12 minutes

to 25-30 minutes was used for assessment of acute tolerance. Chronic tolerance was assessed by comparing the initial N₂O administration for the control group to the 6th N₂O administration for the experimental group and the response variable was the change in threshold from baseline to 7-12 minutes. By disassociating the cue from the drug being administered for some of the subjects during Session 6, it was possible to test for classically conditioned changes in sensory threshold values. Specifically, two-sample t-tests compared groups of subjects receiving different cues but the same drug (either N₂O or placebo). The response variable for these analyses was change in threshold from baseline to 7-12 minutes. All analyses were performed using detection thresholds and then repeated using pain thresholds by the same analytic methods.

3. Results

3.1 Experiment 1: Acute Tolerance and Rebound

Of the 99 subjects who received training in the electrical tooth stimulation procedure, 11 did not participate in the study (i.e., one did not like the rubber dam, one had a new job and could not be scheduled, one had a pain threshold greater than the maximum stimulus intensity of the stimulator, and eight could not be trained to provide consistent / reliable sensory thresholds). Of the remaining 88 subjects, 11 dropped out during the experimental procedures (i.e., one felt nauseous but did not vomit, one vomited, seven began the study but found the experience unpleasant and did not want to continue, one could not attend the second session, and testing was discontinued for one subject due to a blocked nasal cannula). Thus, 77 subjects (38 female / 39 male, mean age = 26.4 years, age range = 18 - 46 years) completed both experimental sessions.

There was a significant increase in average sensory thresholds when N₂O was administered (Fig. 2). The mean change in thresholds from baseline to 7-11 minutes was higher for the N₂O exposure than for the placebo exposure (78.8 ncoul versus 10.1 ncoul for detection threshold, mean difference = 68.7, SE = 7.5, $p < 0.001$; 101.2 ncoul versus 14.3 ncoul for pain threshold, mean difference = 86.9, SE = 7.5, $p < 0.001$). Average baseline thresholds were comparable and not significantly different for N₂O and placebo exposures (63.1 ncoul versus 63.6 ncoul for detection threshold, 117.7 ncoul versus 121.7 ncoul for pain threshold). The drug effect between 7-11 minutes was smaller for detection threshold (68.7 ncoul) than for pain threshold (86.9 ncoul) in absolute terms, but it was a larger fraction of mean baseline thresholds for detection (68.7/63.3) compared with pain (86.9/119.7). Pain thresholds decreased (returned toward baseline values) between 7-11 minutes and 36-40 minutes during the N₂O administration, but not under placebo, indicating that acute tolerance developed to the analgesic effects of N₂O. This difference between N₂O and placebo exposures was statistically significant (decrease of 15.2 ncoul for N₂O, increase of 7.6 ncoul for placebo, mean difference between exposures of 22.8 ncoul, SE = 7.1, $p = 0.002$). However, no evidence for acute tolerance was found using detection thresholds (increase of 0.8 ncoul for N₂O, increase of 5.0 ncoul for placebo, mean difference between exposures of 4.2 ncoul, SE = 8.5, $p = 0.619$).

3.2 Experiment 2: Chronic Tolerance and Associative Processes

Of the 94 subjects who received training in the electrical tooth stimulation procedure, 6 were not able to participate in the study (i.e., four could not be trained to provide consistent / reliable sensory thresholds, one could not be scheduled, one became ineligible for medical reasons). Of the 88 subjects who participated in the actual study, eight were unable to complete the experimental procedures (i.e., one felt nauseous but did not vomit, two vomited, four began the study but found the experience unpleasant and did not want to continue, and testing was discontinued for one subject due to a software problem). Thus, 80 subjects (29 female / 51

male, mean age = 25.5 years, age range = 19 - 41 years) completed all of the experimental sessions.

There was a significant increase in average sensory thresholds when N₂O was administered (Fig. 3). The difference between experimental and control groups in the change from baseline to 7-12 minutes was 58.0 ncoul ($p < 0.001$, 95% CI: 43.7, 72.3) for detection threshold and 63.7 ncoul ($p < 0.001$, 95% CI: 49.0, 78.5) for pain threshold. Although the absolute effect was smaller for detection than for pain, the percentage increase relative to baseline was higher for detection (89%) than for pain (56%). There were no significant differences between groups in baseline thresholds (Detection: 70.4 vs. 74.4, $p > 0.05$; Pain: 121.3 vs. 134.8, $p > 0.05$). Sensory thresholds decreased (returned toward baseline values) from 7-12 minutes to 25-30 minutes during the N₂O administration, but increased slightly during this time period under placebo. The difference between mean changes was statistically significant (detection threshold = 19.1 ncoul, $p = 0.022$, 95% CI: 2.9, 35.3; pain threshold = 22.7 ncoul, $p = 0.001$, 95% CI: 9.1, 36.4) and provides evidence for acute tolerance development.

The within-subjects analysis (Fig. 4) gave similar results to the between-subjects analysis described above. The mean change in thresholds from baseline to 7-12 minutes among experimental group subjects was higher for N₂O exposures than for placebo exposures (difference of 57.0 ncoul, SE = 6.8, for detection threshold, $p < 0.001$; difference of 63.3 ncoul, SE = 6.7 for pain threshold, $p < 0.001$). There were no differences between N₂O and placebo exposures in baseline thresholds for subjects in the experimental group. The mean change in thresholds from 7-12 minutes to 25-30 minutes among experimental group subjects was lower for N₂O exposures than for placebo exposures (difference of 16.6 ncoul for detection threshold, $p = 0.046$, 95% CI: 0.3, 32.9; difference of 17.1 ncoul for pain threshold, $p = 0.012$, 95% CI: 3.8, 30.3).

Chronic tolerance developed for detection threshold (Fig. 5) and pain threshold (Fig. 6). Subjects in the control group had a greater increase in their sensory thresholds during their initial N₂O exposure in Session 6 than subjects in the experimental group during their 6th exposure (difference of 44.1 ncoul for detection threshold, $p = 0.033$, 95% CI: 4.1, 84.1; difference of 36.9 ncoul for pain threshold, $p = 0.026$, 95% CI: 4.9, 68.9).

No evidence was found for classical conditioning. Subjects receiving the identical drug but different cues did not differ significantly in their threshold changes from baseline to 7-12 minutes or from baseline to 25-30 minutes (Table 1).

4. Discussion

4.1 Acute Tolerance and Rebound

A goal of this research was to determine if acute tolerance develops to N₂O. A return of the measured variable toward pre-drug baseline (or control) values during a steady-state drug administration indicates acute tolerance development (Ramchandani et al., 1999; Ramsay and Woods, 1997). N₂O is well suited for research requiring steady-state concentrations. N₂O's low solubility in blood and tissues allows blood levels to equilibrate rapidly with the inspired concentration and to remain constant as long as the gas is administered (Eger II, 1985). The N₂O concentration in the alveoli is estimated to be approximately 90% of the inspired concentration five minutes after the start of administration (Stenqvist, 1994). Furthermore, the lack of human metabolic pathways for N₂O limits dispositional explanations for any tolerance that is observed (Trudell, 1985).

Both experiments indicated that 35% and 38% N₂O cause analgesia in humans and that acute tolerance develops to this effect. This was true for the pain threshold measure in both

experiments and for the detection threshold measure in Experiment 2. In Experiment 1, the drug effect on detection thresholds declined by only 6% between 7-11 minutes (68.7 ncoul) to 36-40 minutes (64.5 ncoul) and this difference was not statistically significant. For pain thresholds, there was a significant decline of 26% from 86.9 ncoul at 7-11 minutes to 64.1 ncoul at 36-40 minutes. The magnitudes of acute tolerance were slightly larger in Experiment 2 and both were statistically significant. The drug effect on detection thresholds declined by 32% (from 57.0 to 38.9 ncoul) and the effect on pain thresholds declined by 34% (from 63.7 to 42.3 ncoul). It is unclear why acute tolerance was not observed on the detection threshold measure from Experiment 1. A common caveat when a study does not observe acute tolerance is that the duration of the drug exposure may have been too brief. However, this is unlikely as Experiment 2 used a 30-min gas administration duration that was 10 min shorter than was used in Experiment 1. The small magnitude of acute tolerance combined with large individual variability may explain why it has been difficult to demonstrate statistically that acute tolerance develops during similarly short exposures (Pirec et al., 1995; Ramsay et al., 1992). Acute tolerance development to N₂O may be easier to document with longer-duration drug exposures (Zacny et al., 1996).

Another goal of this research was to determine whether a hyperalgesic rebound effect occurs following discontinuation of N₂O. Several human studies suggest that rebound effects can occur following N₂O administration. Rupreht and colleagues (1985) described the after-effects experienced by eight subjects after withdrawal from a 3-hr exposure to an anesthetic concentration of N₂O. Nausea occurred in 6 subjects, and all subjects reported feeling cold and having “goose-pimples” on the skin. These after-effects, which gradually disappeared over a 2 to 3-hour period, were likened to an opiate withdrawal syndrome. In other studies, (Henrie et al., 1961; Williams et al., 1984), sub-anesthetic concentrations of N₂O reduced the amplitude of cerebral activity as indexed by an electroencephalographic measure. Upon withdrawal of the drug, the amplitude increased and rebounded above pre-drug control values. The animal literature also provides evidence for N₂O rebound effects (Harper et al., 1980; Manson et al., 1983; Smith et al., 1979b; Stevens et al., 1983). Rupreht and colleagues (1983) proposed that the N₂O withdrawal syndrome might serve as an experimental model for postanesthetic excitation (i.e., emergence delirium) seen in patients following general anesthesia.

The compensatory counter-adaptations that are presumed to be responsible for acute tolerance may also be responsible for rebound. If a drug effect elicits a compensatory reaction, then both the drug effect and the compensatory response jointly influence the dependent measure (Ramsay and Woods, 1997). When drug delivery ceases and the drug concentration falls, there is a relative reduction in the contribution of the drug effect to the dependent measure. If the contribution of the compensatory response dissipates in parallel with the drug effect, no rebound would be observed. However, if the effects caused by the compensatory response continue after the drug and its effects have dissipated, rebound would result. Therefore, the occurrence of rebound is a function of the half-life of the drug effect relative to the rate at which the effects of the compensatory response dissipate. The appearance and severity of rebound phenomena depend greatly upon the speed with which the drug concentration drops following the termination of drug delivery (Lupolover et al., 1982). Given the development of acute tolerance on the pain threshold measure, a hyperalgesic rebound effect might be expected, especially considering the quick elimination of N₂O. Although the pain threshold values returned more fully to control levels than did the detection threshold values, there was no evidence of a hyperalgesic rebound effect following discontinuation of the N₂O administration.

4.2 Chronic Tolerance and Associative Processes

The primary goal of Experiment 2 was to determine whether chronic tolerance develops to 35% N₂O analgesia. The present results indicate that chronic tolerance develops to N₂O

analgesia. Within-subject comparisons indicate that subjects experience less analgesia from N₂O during a sixth exposure than during an initial exposure (Figs. 5 and 6). By the sixth N₂O exposure, the magnitude of the analgesic effect on detection and pain thresholds was significantly reduced by 45% (from 57.0 to 31.1 ncoul) and 39% (from 63.2 to 38.3 ncoul) respectively, compared with the initial exposure. In addition, the magnitude of N₂O analgesia exhibited by control subjects who received N₂O for the first time during the final session rules out the interpretation that chronic tolerance might develop as an artifact of the experimental procedures per se.

A secondary goal of Experiment 2 was to investigate whether associative learning contributed to chronic tolerance development. An influential model of drug tolerance (Siegel et al., 2000) suggests that tolerance results from activation of classically conditioned responses that compensate for the drug's effect. By incorporating a Pavlovian drug conditioning procedure into the experimental design of the chronic tolerance study, it was possible to test two hypotheses derived from an associative model of tolerance. One hypothesis was that chronically tolerant subjects given a drug predictive cue but who are administered a placebo gas would exhibit a conditioned response of increased pain sensitivity (Siegel, 1983). The other hypothesis was that chronically tolerant subjects given a placebo predictive cue but who receive N₂O would not appear tolerant (i.e., would exhibit environmentally specific tolerance) (Siegel and MacRae, 1984). However, neither hypothesis was supported, as there was no evidence of classically conditioned changes in sensitivity to tooth stimulation even though chronic tolerance had developed. It is difficult to interpret null findings and there are a myriad of possible reasons why classical conditioning was not observed. For example, the same experimenter, dental chair and operatory were used for all trials regardless of whether placebo or N₂O was administered and thus there may have been stimulus generalization among environmental cues. It is difficult to know whether the putative cues were sufficiently salient or if there were an adequate number of conditioning trials to learn the discrimination.

A possible explanation for the failure to observe evidence of conditioned tolerance is that the initial interoceptive effects of N₂O provide a potent drug onset cue that signals upcoming drug effects (Greeley et al., 1984; Greeley and Ryan, 1995; Kim et al., 1999; Siegel et al., 2000). For example, the concentration of N₂O used in the present research quickly and dramatically alters one's sensorium. The presence of this interoceptive effect (an internal state of the organism) may be more salient and a better predictor of impending drug effects than are the exteroceptive cues (e.g., a distinct light, sound, and odor) that were experimentally paired with the drug. Thus, interoceptive drug cues may overshadow the simultaneously presented environmental cues (Siegel et al., 2000). Finally, of course, and in contrast to what occurs with other drugs (Siegel et al., 2000), conditioning processes may not contribute to the development of chronic tolerance to N₂O's analgesic effect.

Large individual differences were observed with respect to N₂O's analgesic effect. This aspect of the data is not obvious from an examination of the averaged sensory threshold data. Some individuals appeared initially insensitive to N₂O analgesia, while others had a large analgesic effect that was maintained throughout the N₂O administration, and still others demonstrated a recovery of their sensory thresholds despite the continuous administration of N₂O (i.e., became acutely tolerant). Large individual differences are often noted in human research with N₂O (Benedetti et al., 1982; Dohrn, et al., 1993; Dworkin et al., 1983; Kaufman et al., 1992; Ramsay et al., 1992). From a clinical perspective, it is important to appreciate that there are large individual differences in initial sensitivity and acute tolerance. For example, while there is only a small average reduction in analgesic effectiveness due to acute tolerance development, the effect can be quite large for some individuals. Interestingly, animal research indicates that individual differences in initial sensitivity and acute tolerance development to N₂O's hypothermic effects are reliable characteristics of the individual (Kaiyala, et al., 2001).

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REFERENCE LIST

1. Armitage, P.; Berry, G.; Matthews, JNS. Statistical methods in medical research. 4th edition. Blackwell Science; Oxford; Malden, MA: 2002.
2. Avramov MN, Shingu K, Mori K. Progressive changes in electroencephalographic responses to nitrous oxide in humans: A possible acute drug tolerance. *Anesth Analg* 1990;70:369–74. [PubMed: 2316879]
3. Benedetti C, Chapman, CR, Colpitts YH, Chen ACN. Effects of nitrous oxide concentration on event-related potentials during painful tooth stimulation. *Anesthesiology* 1982;56:360–64. [PubMed: 7072999]
4. Berkowitz BA, Finck AD, Hynes MD, Ngai SH. Tolerance to nitrous oxide analgesia in rats and mice. *Anesthesiology* 1979;51:309–12. [PubMed: 484891]
5. Berkowitz BA, Finck AD, Ngai SH. Nitrous oxide analgesia: Reversal by naloxone and development of tolerance. *J Pharmacol Exp Ther* 1977;203:539–47. [PubMed: 925957]
6. Brown AC, Beeler WJ, Kloka AC, Fields RW. Spatial summation of pre-pain and pain in human teeth. *Pain* 1985;21:1–16. [PubMed: 3982834]
7. Brown AC, Kloka AC, Beeler WJ. Sensory response to constant charge stimulation of teeth. *J Dent Res* 1984;63:203.
8. Champion MA, Kugel G, Gruskowski C. Evaluation of a new intraoral isolation device. *Oper Dent* 1991;16:181–5. [PubMed: 1813873]
9. Dohrn CS, Lichtor JL, Coalson DW, Uitvlugt A, de Wit H, Zacny JP. Reinforcing effects of extended inhalation of nitrous oxide in humans. *Drug Alcohol Depend* 1993;31:265–80. [PubMed: 8462415]
10. Dworkin SF, Chen ACN, Schubert MM, Clark DW. Analgesic effects of nitrous oxide with controlled painful stimuli. *J Am Dent Assoc* 1983;107:581–5. [PubMed: 6579091]
11. Dzoljic M, Rupprecht J, Erdmann W, Stijnen TH, Briemen LJ, Dzoljic MR. Behavioral and electrophysiological aspects of nitrous oxide dependence. *Brain Res Bull* 1994;33:25–31. [PubMed: 8275324]
12. Eger, EI, II. MAC. In: Eger, EI, II, editor. Nitrous oxide / N₂O. Elsevier Science Publishing Company, Inc.; New York: 1985. p. 57–67.
13. Fender C, Fujinaga M, Maze M. Strain differences in the antinociceptive effect of nitrous oxide on the tail flick test in rats. *Anesth Analg* 2000;90:195–9. [PubMed: 10625003]
14. Finck, AD. Nitrous oxide analgesia. In: Eger, EI, II, editor. Nitrous oxide / N₂O. Elsevier Science Publishing Company, Inc.; New York: 1985. p. 41–55.
15. Gracely RH, Lota L, Walter DJ, Dubner R. A multiple random staircase method of psychophysical pain assessment. *Pain* 1988;32:55–63. [PubMed: 3340422]
16. Greeley J, Lê DA, Poulos CX, Cappell H. Alcohol is an effective cue in the conditional control of tolerance to alcohol. *Psychopharmacology (Berl)* 1984;83:159–62. [PubMed: 6431466]
17. Greeley, J.; Ryan, C. The role of interoceptive cues for drug delivery in conditioning models of drug dependence. In: Drummond, DC.; Tiffany, ST.; Gledtier, S.; Remington, B., editors. Addictive Behaviour: Cue Exposure Theory and Practice. John Wiley & Sons, Ltd.; Chichester England: 1995. p. 119–36.
18. Harper MH, Winter PM, Johnson BH, Koblin DD, Eger EI II. Withdrawal convulsions in mice following nitrous oxide. *Anesth Analg* 1980;59:19–21. [PubMed: 7188661]
19. Haugen FP, Coppock WJ, Berquist HC. Nitrous oxide hypalgesia in trained subjects. *Anesthesiology* 1959;20:321–4. [PubMed: 13650216]

20. Henrie JR, Parkhouse J, Bickford RG. Alteration of human consciousness by nitrous oxide as assessed by electroencephalography and psychological tests. *Anesthesiology* 1961;22:247–59. [PubMed: 13713391]
21. Kaiyala KJ, Leroux BG, Watson CH, Prall CW, Coldwell SE, Woods SC, Ramsay DS. Reliability of individual differences in initial sensitivity and acute tolerance to nitrous oxide hypothermia. *Pharmacol Biochem Behav* 2001;68:691–9. [PubMed: 11526966]
22. Kalant H, LeBlanc AE, Gibbins RJ. Tolerance to, and dependence on, some non-opiate psychotropic drugs. *Pharmacol Rev* 1971;23:135–91. [PubMed: 4398655]
23. Kaufman E, Chastain DC, Gaughan AM, Gracely RH. Staircase assessment of the magnitude and time-course of 50% nitrous-oxide analgesia. *J Dent Res* 1992;71:1598–603. [PubMed: 1522292]
24. Kim JA, Siegel S, Patenall VR. Drug-onset cues as signals: intradiministration associations and tolerance. *J Exp Psychol Anim Behav Process* 1999;25:491–504. [PubMed: 10531660]
25. Korttila K, Ghoneim MM, Jacobs L, Mewaldt SP, Petersen RC. Time course of mental and psychomotor effects of 30 per cent nitrous oxide during inhalation and recovery. *Anesthesiology* 1981;54:220–6. [PubMed: 7469105]
26. Lupolover R, Dazzi H, Ward J. Rebound phenomena: Results of a 10 year's (1970-1980) literature review. *Int Pharmacopsychiatry* 1982;17:194–237. [PubMed: 6136487]
27. Manson HJ, Dyke G, Melling J, Gough M. The effect of naloxone and morphine on convulsions in mice following withdrawal from nitrous oxide. *Can Anaesth Soc J* 1983;30:28–31. [PubMed: 6297697]
28. Maze M, Fujinaga M. Recent advances in understanding the actions and toxicity of nitrous oxide. *Anaesthesia* 2000;55:311–4. [PubMed: 10781114]
29. Moore PA. Psychomotor impairment due to N₂O exposure. *Anesth Prog* 1983;30:72–5. [PubMed: 6580828]
30. Persson P. Nitrous oxide hypalgesia in man. *Acta Odontol Scand* 1951;9:1–98.
31. Pirec V, Patterson TH, Thapar P, Apfelbaum JL, Zacny JP. Effects of subanesthetic concentrations of nitrous oxide on cold-pressor pain in humans. *Pharmacol Biochem Behav* 1995;51:323–9. [PubMed: 7667348]
32. Quock RM, Vaughn LK. Nitrous oxide: Mechanism of its antinociceptive action. *Analgesia* 1995;1:151–9.
33. Ramchandani VA, O'Connor S, Blekher T, Kareken D, Morzorati S, Nurnberger J, Li T-K. A preliminary study of acute responses to clamped alcohol concentration and family history of alcoholism. *Alcohol: Clin Exp Res* 1999;23:1320–30. [PubMed: 10470974]
34. Ramsay DS, Brown AC, Woods SC. Acute tolerance to nitrous oxide in humans. *Pain* 1992;51:367–73. [PubMed: 1491863]
35. Ramsay DS, Omachi K, Leroux BG, Seeley RJ, Prall C, Woods SC. Nitrous oxide-induced hypothermia in the rat: Acute and chronic tolerance. *Pharmacol Biochem Behav* 1999;62:189–96. [PubMed: 9972863]
36. Ramsay DS, Woods SC. Biological consequences of a drug administration: Implications for acute and chronic tolerance. *Psychol Rev* 1997;104:170–93. [PubMed: 9009884]
37. Ruprecht J, Dworacek B, Ducardus R, Schmitz PI, Dzoljic MR. The involvement of the central cholinergic and endorphinergic systems in the nitrous oxide withdrawal syndrome in mice. *Anesthesiology* 1983;58:524–6. [PubMed: 6683088]
38. Ruprecht J, Ukponmwan EO, Dworacek B, Admiraal PV, Dzoljic MR. Enkephalinase inhibition prevented tolerance to nitrous oxide analgesia in rats. *Acta Anaesthesiol Scand* 1984;28:617–20. [PubMed: 6395608]
39. Ruprecht J, Dworacek B, Bonke B, Dzoljic MR, van Eijndhoven JHM, de Vlieger M. Tolerance to nitrous oxide in volunteers. *Acta Anaesthesiol Scand* 1985;29:635–8. [PubMed: 4061009]
40. Shingu K, Osawa M, Fukuda K, Mori K. Acute tolerance to the analgesic action of nitrous oxide does not develop in rats. *Anesthesiology* 1985;62:502–4. [PubMed: 3985406]
41. Siegel S. Classical conditioning, drug tolerance, and drug dependence. In: Israel, Y.; Glaser, FB.; Kalant, H.; Popham, RE.; Schmidt, W.; Smart, RG., editors. *Research Advances in Alcohol and Drug Problems*. Plenum Press; New York: 1983. p. 207–46.

42. Siegel S, Baptista MA, Kim JA, McDonald RV, Wiese-Kelly L. Pavlovian psychopharmacology: the associative basis of tolerance. *Exp Clin Psychopharmacol* 2000;8:276–93. [PubMed: 10975617]
43. Siegel S, MacRae J. Environmental specificity of tolerance. *Trends Neurosci* 1984;7:140–2.
44. Smith RA, Winter PM, Smith M, Eger EI II. Rapidly developing tolerance to acute exposures to anesthetic agents. *Anesthesiology* 1979a;50:496–500. [PubMed: 453575]
45. Smith RA, Winter PM, Smith M, Eger EI II. Convulsions in mice after anesthesia. *Anesthesiology* 1979b;50:501–4. [PubMed: 36819]
46. Stenqvist O. Nitrous oxide kinetics. *Acta Anaesthesiol Scand* 1994;38:757–60. [PubMed: 7887090]
47. Stevens JE, Oshima E, Mori K. Effects of nitrous oxide on the epileptogenic property of enflurane in cats. *Brit J Anaesth* 1983;55:145–54. [PubMed: 6830677]
48. Trudell, JR. Metabolism of nitrous oxide. In: Eger, EI., II, editor. *Nitrous oxide / N₂O*. Elsevier Science Publishing Company, Inc.; New York: 1985. p. 203-10.
49. Whitwam JG, Morgan M, Hall GM, Petrie A. Pain during continuous nitrous oxide administration. *Br J Anaesth* 1976;48:425–8. [PubMed: 1276015]
50. Williams DJM, Morgan RJM, Sebel PS, Maynard DE. The effect of nitrous oxide on cerebral electrical activity. *Anaesthesia* 1984;39:422–5. [PubMed: 6731769]
51. Yajnik S, Zacny JP, Young CJ, Lichtor JL, Rupani G, Klafta JM, Coalson DW, Apfelbaum JL. Lack of acute tolerance development to the subjective, cognitive, and psychomotor effects of nitrous oxide in healthy volunteers. *Pharmacol Biochem Behav* 1996;54:501–508. [PubMed: 8743615]
52. Zacny JP, Cho AM, Coalson DW, Rupani G, Young CJ, Klafta JM, Klock PA, Apfelbaum JL. Differential acute tolerance development to effects of nitrous oxide in humans. *Neurosci Lett* 1996;209:73–76. [PubMed: 8761985]

Fig. 1.

The top panel describes the procedures used during the first 5 test sessions and the bottom panel describes the procedures used during the 6th session of Experiment 2. Time (min) is provided on the axis, and the episodes of sensory threshold assessment are indicated by the arrows pointing at the axis. The shaded bars identify when the nitrous oxide (N₂O) or placebo (P) gas were administered.

Figure 2.

Mean change in sensory thresholds between the comparable assessment intervals during the N₂O and placebo sessions (N = 77) of Experiment 1. Sensory thresholds increased during inhalation of 38% N₂O and acute tolerance developed for the pain threshold difference score but not for the detection threshold difference score. The box indicates when the 38% N₂O was administered during the N₂O session.

Fig. 3. Mean sensory thresholds and standard error bars for Experiment 2. Subjects inhaling 35% N₂O had increased sensory thresholds during the 30-min exposure. Control subjects received placebo gas during the entire session. Acute tolerance developed for both sensory threshold measures.

Fig. 4.

Mean change in sensory thresholds between the comparable assessment intervals during the first session's N₂O and placebo exposures (N = 64) of Experiment 2. Sensory thresholds increased during inhalation of 35% N₂O and acute tolerance developed for both the pain threshold difference score and the detection threshold difference score. The box indicates when the 35% N₂O was administered during the N₂O session.

Fig. 5.

Mean change (standard error bars) in detection threshold from the 7-12 minute assessment minus the pre-drug baseline value for Experiment 2. During the 1st session, N₂O administered to the experimental group increased detection threshold relative to a placebo control group. During the 6th session, control subjects who received N₂O for the first time had a greater change in detection threshold than did experimental group subjects who received N₂O for the sixth time.

Fig. 6.

Mean change (standard error bars) in pain threshold from the 7-12 minute assessment minus the pre-drug baseline value for Experiment 2. During the first session, N₂O administered to the experimental group increased pain threshold relative to a placebo control group. During the sixth session, control subjects who received N₂O for the first time had a greater change in pain threshold than did experimental group subjects who received N₂O for the sixth time. [Some subjects had indeterminate pain threshold values.]

Table 1.

Difference in sensory thresholds (ncoul) from baseline values during the initial gas exposure of Session 6 of Experiment 2. There was no evidence that providing the drug cue independently from the drug being administered influenced sensory thresholds. Subjects assigned to the drug groups breathed N₂O from 0-30 minutes while subjects assigned to the placebo groups breathed the placebo gas from 0-30 minutes.

Drug Assignment	Cue Assignment	Assessment Interval (min)	Change Detection Threshold	Change Pain Threshold
			Mean (N, SEM, Range)	Mean (N, SEM, Range)
Placebo	Placebo	7 - 12	11.0 (16, 3.5, -13.2 - 33.0)	7.3 (16, 3.5, -21.0 - 35.5)
Placebo	Nitrous Oxide	7 - 12	14.1 (16, 4.3, -1.8 - 52.0)	13.2 (16, 5.2, -23.0 - 55.5)
Nitrous Oxide	Nitrous Oxide	7 - 12	39.4 (16, 7.9, -3.3 - 120.0)	52.5 (15, 13.1, -10.5 - 162.5)
Nitrous Oxide	Placebo	7 - 12	34.4 (16, 8.9, -9.0 - 153.0)	46.2 (15, 10.9, -15.5 - 162.0)
Placebo	Placebo	25 - 30	21.5 (16, 4.0, 0.0 - 52.5)	21.2 (16, 5.4, -4.5 - 75.0)
Placebo	Nitrous Oxide	25 - 30	14.4 (16, 3.0, -1.8 - 41.2)	29.7 (16, 13.0, -9.5 - 213.5)
Nitrous Oxide	Nitrous Oxide	25 - 30	58.4 (15, 15.9, -50.5 - 156.5)	54.4 (14, 13.7, -12.0 - 137.5)
Nitrous Oxide	Placebo	25 - 30	36.4 (15, 10.5, -43.5 - 128.0)	46.6 (15, 6.9, 7.5 - 99.5)