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Nitrous Oxide-Induced Hypothermia in the Rat: Acute and Chronic Tolerance

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RAMSAY, D. S., K. OMACHI, B. G. LEROUX, R. J. SEELEY, C. W. PRALL AND S. C. WOODS. *Nitrous oxide-induced hypothermia in the rat: Acute and chronic tolerance.* PHARMACOL BIOCHEM BEHAV **62**(1) 189–196, 1999.—Although inhalation of nitrous oxide (N_2O) causes hypothermia in rats, there is a paucity of information as to whether tolerance develops to this effect. The purpose of this study was to determine whether tolerance to N_2O hypothermia develops within a single administration as well as over repeated administrations. Temperature was measured telemetrically by implanting intraperitoneal thermal sensors/transmitters in male Long–Evans rats. Experimental rats received an initial 2-h exposure to 60% N_2O and became hypothermic relative to controls breathing placebo gas. Only a few rats demonstrated evidence of acute tolerance over the 120 min. Over the next 10 days, the experimental rats received five additional 30-min exposures to 60% N_2O and five 30-min exposures to placebo while the control rats received only placebo gas exposures. Chronic tolerance developed to N_2O hypothermia over these repeated administrations. A test for Pavlovian drug conditioning found no evidence that conditioned temperature effects contributed to chronic tolerance development. In a second experiment, naive rats were given a 380-min exposure to 60% N_2O and a 380-min exposure to placebo gas in a counterbalanced order. Acute tolerance did develop to N_2O hypothermia, with the recovery of temperature beginning after a mean of 141 min of gas administration. Hence, both acute and chronic tolerance develop to N_2O's hypothermic effects in rats. © 1998 Elsevier Science Inc.

Pavlovian drug conditioning Individual differences

NITROUS oxide (N_2O) is a pharmacologically active gas. At subanesthetic concentrations, N_2O is best known for its ability to diminish the pain and anxiety patients may experience while undergoing stressful medical or dental procedures. When administered at hyperbaric concentrations, N_2O produces general anesthesia (34). Although the clinical use of N_2O is widespread, little is known about the development of tolerance to the drug. This is important, because the development of drug tolerance can diminish the efficacy of a drug both during a single administration (i.e., acute tolerance) as well as over repeated administrations (i.e., chronic tolerance) [e.g., (31)].

Research on humans suggests that tolerance develops to N_2O when it is administered at anesthetic concentrations (33), and when it is simultaneously administered during anesthesia with other more potent anesthetic agents (1). At subanesthetic concentrations, acute tolerance to N_2O's analgesic effect has been reported to develop in a subset of subjects [e.g., (29,43)], while other investigators have observed no evidence of its development (26,46). Investigations of N_2O's subjective, cognitive, and psychomotor effects in humans have not provided evidence for acute tolerance development (19,24,47). A recent study (48) investigated the analgesic, subjective, and psychomotor effects of N_2O and found that acute tolerance develops to some, but not all, of these effects. Specifically, acute tolerance developed for analgesia and for subjective effects that were hedonic in nature but not for the other dependent measures.

Research investigating the phenomenon of drug tolerance in animals has also been conducted with N_2O. With respect to N_2O analgesia, tolerance has been reported in some (3,4,32), but not all, animal studies (37). Tolerance to N_2O has been

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documented for nonanalgesic measures that include locomotor activity, visual evoked potentials, and the righting reflex (9,41).

N_2O provides advantages for the investigation of tolerance development. N_2O'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 (10). The ease with which steady-state drug concentrations can be achieved and maintained facilitates the assessment of acute tolerance development, because declining drug effects can be measured independently of changes in the drug concentration (31). Furthermore, the lack of metabolic pathways for N_2O limits dispositional explanations for any tolerance that is observed (42).

The purpose of the present study was to investigate the development of acute and chronic tolerance to N_2O's hypothermic effects. Subanesthetic concentrations of N_2O have a hypothermic effect in rats (28) and mice (25). Body temperature is a homeostatically regulated physiological parameter that lends itself to accurate and continuous noninvasive measurement. Because of this, body temperature is a commonly used dependent measure in the study of drug tolerance [e.g., (14, 20,23)]. The present study also evaluated the development of chronic tolerance within the context of a Pavlovian conditioning procedure (5) to assess whether associative processes may play a role in the expression of tolerance to the drug's hypothermic effect.

GENERAL METHODS

Subjects

Male Long–Evans rats, reared in the vivarium of the Department of Psychology at the University of Washington, were individually maintained in a temperature (25 ± 2°C)- and light (12-L:12-D cycle)-controlled room in standard hanging stainless steel cages. They were provided ad lib food (pelleted chow) and water in their home cages. They ranged in weight from 270–402 g at the initiation of these studies.

Apparatus

Two specialized types of apparatus were used. One was a telemetric temperature measurement system, and the other was a customized gas/odor delivery system.

Telemetric temperature assessment was accomplished using sensors purchased from the Mini-Mitter Company (Sun River, OR). The receiver for the temperature signal was built in our lab following the method described by Cunningham and Peris (6). Each transmitter (i.e., sensor) was calibrated locally using a constant temperature water bath. Temperature data were collected and analyzed using a Macintosh IIci computer and LabVIEW software from National Instruments (Austin, TX).

To deliver a placebo gas, a commercially available N_2O and oxygen (O_2) delivery unit (Nitrox Inc., Woodinville, WA) was modified so that turning a valve could substitute the delivery of nitrogen (N_2) for N_2O while holding the O_2 concentration constant. Subjects receiving N_2O/O_2 were administered 60% N_2O and 40% O_2; subjects receiving placebo were given 60% N_2 and 40% O_2. The concentration of the gas mixture delivered to the testing chamber was verified by use of an infrared gas analyzer (Datex model #CD202; Helsinki, Finland). Gas was delivered simultaneously to four separate Plexiglas enclosures (18 × 19 × 12.5 cm), each with its own independent flowmeter. Gas was delivered to each chamber at a flow rate of 7 liters/min. Finally, to provide an odor cue in association with the gas being delivered, a manual valve could be turned that diverted the gas stream to each chamber through a bubble-through respiratory gas humidifier (Hudson Oxygen Therapy Sales Co., Temecula, CA) that contained plain water for nonodorized gas or a humidifier that contained 105 ml of water mixed with either 3.5 ml of banana extract or 3.5 ml of almond extract. Thus, the gas delivered to each chamber could be nonodorized or odorized with either the odor of banana or almond.

Surgical Procedure

Calibrated temperature sensors were placed surgically into a rat's peritoneal cavity at least 1 week prior to data collection. Halothane anesthesia was used to perform the surgery, and each animal received a 0.1 ml IM injection of 10% (w/v) chloromycetin sodium succinate following surgery.

Data Analysis

A temperature value was recorded for each rat once every 6 s. Thus, there were 30 data points collected for each rat during a 3-min period. A median value was then determined for each 3-min period and this value was used in the subsequent analyses.

EXPERIMENT 1: CHRONIC TOLERANCE

Materials and Methods

Subjects. Initially, 40 male rats were divided into an experimental group ($n = 32$; eight squads of four rats per squad), and a control group ($n = 8$; two squads of four rats per squad). However, problems with the gas delivery system midway through the testing of two different squads (one experimental and one control squad) necessitated replacement of these squads to maintain the experimental design. Therefore, a total of 48 male rats began the experiment ($n = 36$ for the experimental group and $n = 12$ for the control group), although only 40 completed the entire experimental protocol.

Procedure. All rats were given a 30-min familiarization exposure to the gas exposure chambers once a day for 3 days prior to the start of the experimental trials. The N_2/O_2 gas mixture was delivered on these trials. During the experiment, rats were administered gas individually in the gas exposure chambers, and each rat was always tested in the same gas exposure chamber. All treatment assignments were made randomly by squad. All members of a squad received the same drug and odor, and were run concurrently. All gas administrations began at least 2.5 h after the start of the light phase, and always concluded no later than 2.5 h prior to the start of the dark phase.

On day 1, rats in the experimental group were removed from their home cage, placed into the gas exposure chamber (in an adjacent room), and given a 120-min administration of odorized (i.e., almond or banana) N_2O (60% N_2O and 40% O_2), followed by 150 min of nonodorized placebo gas (60% N_2 and 40% O_2). Control rats received a 120-min administration of odorized (i.e., almond or banana) placebo gas followed by 150 min of nonodorized placebo gas. After the 270-min trial, both experimental and control rats were returned to their home cages. The design was counterbalanced in that a randomly selected half of the experimental and control squads received almond during the 120-min initial period, and the remaining squads received banana.

On days 2–11, the daily gas administration lasted for 30 min. Rats in the experimental group received exposure to either odorized placebo or odorized N_2O on each day. For these experimental squads, each dyad of days (i.e., days 2–3, 4–5, 6–7, 8–9, and 10–11) included one placebo and one N_2O exposure, the order of drug administrations being determined randomly for each dyad. Each rat in the experimental group received the same odor with N_2O on days 2–11 as on the day 1 exposure, and the other odor (i.e., almond or banana) was administered in association with the placebo gas. Rats in the control group received odorized placebo gas on days 2–11, and the order of the odors was determined randomly within each dyad.

On day 12, the eight experimental squads of rats were randomly subdivided into four equal groups of two squads each. The experimental protocol on day 12 resembled the procedure used on the day 1 in that there was an initial 120-min administration of odorized gas followed by a 150-min administration of nonodorized placebo gas. The four subgroups of experimental rats differed only during the 120-min exposure to the odorized gas as follows: (a) 60% N_2O and 40% O_2 with the same odor that had been paired with N_2O, (b) 60% N_2O and 40% O_2 with the odor that had been paired with placebo gas administrations, (c) 60% N_2 and 40% O_2 with the odor that had been previously paired with placebo gas, and (d) 60% N_2 and 40% O_2 with the odor that had been paired with N_2O. The control rats received N_2O (60% N_2O and 40% O_2) for the first time in the experiment during the 120-min exposure, which was followed by a 150-min exposure to nonodorized placebo gas. The odor that was delivered during the 120 min N_2O exposure for the control rats was the same as each animal received during the day 1 gas exposure.

Data analysis. The effect of initial exposure to N_2O was assessed by comparing experimental ($n = 36$) and control ($n = 12$) groups on the maximum temperature reduction from baseline during the 120-min gas exposure on day 1, using a two-sample *t*-test. Because the odors were not completely counterbalanced due to the replacement of squads following an equipment malfunction, this test was repeated using only the 10 counterbalanced squads. In addition, animals receiving almond and banana odors on day 1 were compared using separate *t*-tests for the experimental and control groups to verify that the specific odors had no effect on temperature. Because there were no differences between the two odors, all subsequent analyses were conducted using the data from the 10 counterbalanced squads. The development of chronic tolerance was assessed by comparing the absolute difference in the average temperature during the 30-min gas exposure between consecutive days within each dyad (i.e., days 2–3, 4–5, 6–7, 8–9, 10–11). Repeated-measures analysis of variance was applied to these values, and the test of chronic tolerance was based on the multivariate test of the interaction between group (experimental vs. control) and time (dyads 1–5). On day 12, chronic tolerance was also assessed by comparing the effect of N_2O in the control group receiving the drug for the first time ($n = 8$) with the effect on those experimental animals receiving N_2O with the same odor cue that was paired with N_2O on days 1–11 ($n = 8$). The maximum temperature reduction from baseline during the 120-min gas exposure was compared for the two groups by a two-sample *t*-test. Assessments of environmentally specific changes were made by comparing groups of animals receiving the same drug but different odor cues on day 12, using the maximum temperature reduction from baseline during the 120-min gas exposure, by two-sample *t*-tests. All tests initially ignored squad effects, but were subsequently verified by including a random squad effect to account for intrasquad correlation where appropriate.

Results

Temperature data from all 48 rats are included in the analysis of the day 1 gas exposure. Inclusion of the data from the two additional squads does not provide a completely counterbalanced exposure to the two different odors on day 1. However, the day 1 results obtained using the data from only the counterbalanced squads were very similar. Furthermore, specific odor was not found to have an effect on body temperature during the day 1 placebo or N_2O administration. The results of the day 1 gas exposure are shown in Fig. 1. The experimental and control groups did not differ at baseline [placebo = 37.7 and N_2O = 37.4; $t(46) = 1.35$; $p > 0.05$]. Both groups decreased their mean temperature during the first 120 min of gas exposure, and the two groups differed in their average reduction [placebo = 0.8 and N_2O = 2.6; $t(46) = 4.78$, $p < 0.001$]. Sixty percent N_2O therefore had a robust hypothermic effect. On a statistical basis, acute tolerance was not apparent to the hypothermic effects of N_2O over the course of its 120-min administration. However, as discussed below, considerable individual differences were observed to the hypothermic effects of N_2O.

In subsequent analyses, only the data collected from the 10 counterbalanced squads that completed all of the experimental sessions were included. Data from two rats in the experimental group were excluded because of missing data resulting from equipment problems. Over days 2–11, chronic tolerance developed to the hypothermic effects of N_2O. Figure 2 depicts the mean temperatures of the experimental and control groups on days 2–11. While Fig. 2 includes all of the data from days 2–11, a clearer illustration of the development of chronic tolerance is provided in Fig. 3. The absolute temperature difference was calculated for each rat between consecutive days within each dyad (i.e., days 2–3, 4–5, 6–7, 8–9, and 10–11) which, for rats in the experimental group, contains one N_2O and one placebo exposure. It is clear from Fig. 3 that the temperature difference between N_2O and placebo exposures lessened with repeated N_2O exposure, indicating the develop-

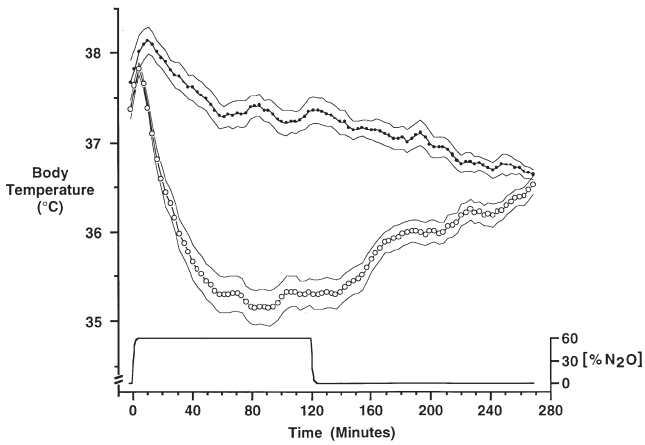

FIG. 1. Mean body temperature and standard error bands. Experimental rats (open circles; $n = 36$) became hypothermic during an initial 120-min exposure to 60% N_2O. Control rats (closed circles; $n = 12$) received placebo gas during the entire 270-min session.

ment of chronic tolerance to N_2O hypothermia, $F(4, 33) = 2.74$, $p < 0.05$.

The inclusion of a separate placebo control condition allowed verification that simply participating in the experimental procedures per se did not result in a reduced hypothermic effect to N_2O. We compared the mean maximum reduction of body temperature between days 1 and 12 for the subset of the experimental rats ($n = 8$) that had received multiple N_2O exposures with a consistent odor pairing vs. the control rats ($n = 8$) that were receiving N_2O for the first time. Figure 4 illustrates that the control and experimental rats experienced a comparable hypothermic effect on initial exposure to N_2O (day 1 for experimental rats vs. day 12 for control rats). The experimental rats experienced significantly less hypothermia during N_2O on day 12 (mean temperature reduction = 0.9°C, SEM = 0.3) than did the control rats on day 12 (mean temperature reduction = 1.9°C, SEM = 0.3); i.e., chronic tolerance developed to N_2O in the experimental rats, $t(14) = 2.41$, $p < 0.05$.

By disassociating the odor cue from the drug being administered to some of the experimental groups on day 12, we attempted to assess whether any classically conditioned changes of body temperature could be observed. These data are depicted in Fig. 5. Groups of rats receiving the identical drug but different cues did not differ in their temperature reductions [placebo drug, $t(12) = 0.56$, $p > 0.05$; N_2O drug, $t(14) = 0.24$, $p > 0.05$].

Discussion

The present results are consistent with other reports demonstrating that N_2O causes hypothermia in rats (28). Given the parameters used in the present experiment, the mean drop in temperature on the first exposure to N_2O was approximately 2°C. However, during 120 min of N_2O administration, there was no mean recovery of the degree of hypothermia, suggesting that acute tolerance had not developed. One possible explanation is that acute tolerance does not develop to N_2O in this paradigm. However, because several individual rats appeared to develop acute tolerance, it may be that the time of drug exposure (120 min) was too short to enable tolerance to develop in most animals.

When rats were exposed to N_2O over several trials, chronic tolerance was seen to develop (Figs. 2–4). However, there was no evidence that the odor that had been associated with N_2O administration was important to the magnitude of the change of temperature given the experimental parameters.

EXPERIMENT 2: ACUTE TOLERANCE

Experiment 1 did not find statistical evidence for the development of acute tolerance over the course of a single 120-min administration of 60% N_2O. Therefore, in Experiment 2 the duration of the exposure to 60% N_2O was increased to 380 min.

Materials and Methods

Subjects. Eight male rats (two squads of four rats per squad) were tested in this experiment.

Procedure. As in the first experiment, all rats were given three 30-min familiarization sessions in the gas exposure chambers prior to the start of the experimental trials. All gas administrations began at least 2.5 h after the start of the light phase, and always concluded no later than 2.5 h prior to the start of the dark phase.

Both squads of rats received two 380-min administrations of nonodorized gas on two separate days. One squad of rats

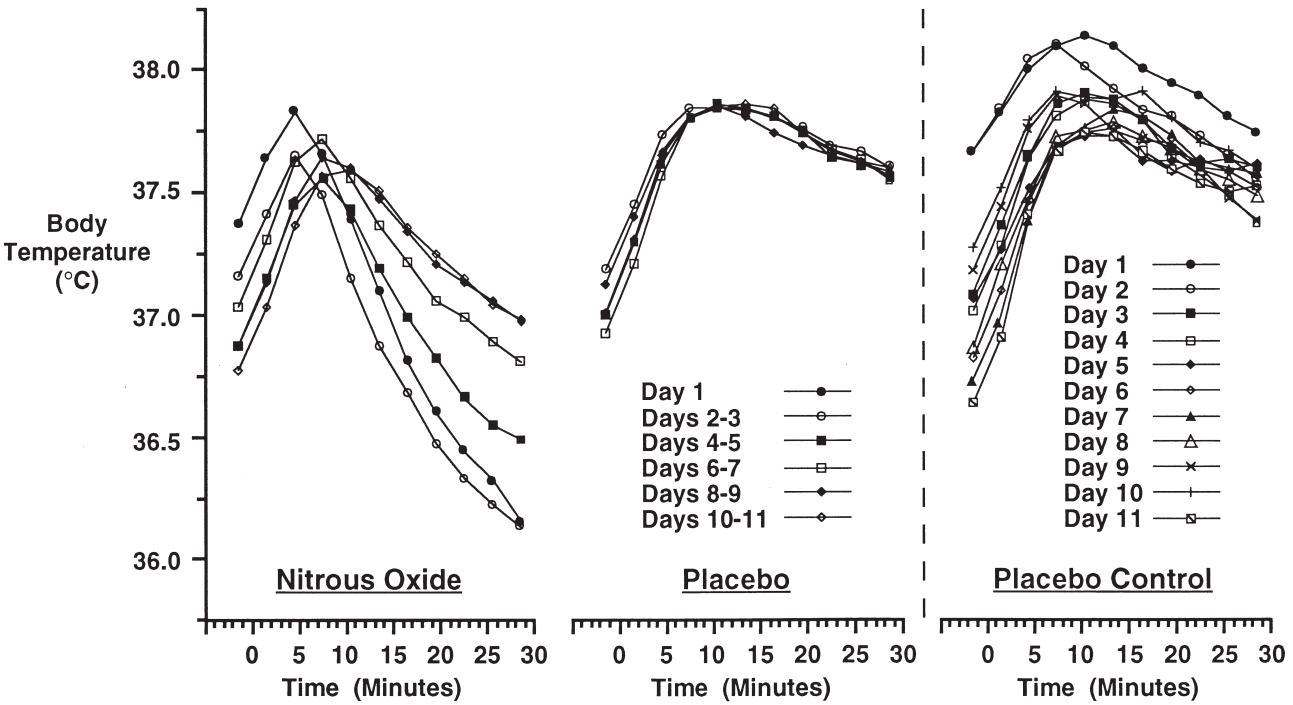

FIG. 2. Mean body temperature over days 1–11 for experimental (left and middle panels) and control (right panel) animals. Chronic tolerance develops over six separate N_2O exposures (left and middle panels; $n = 32$), which is in contrast to the temperature variation observed in the placebo control group (right panel; $n = 8$).

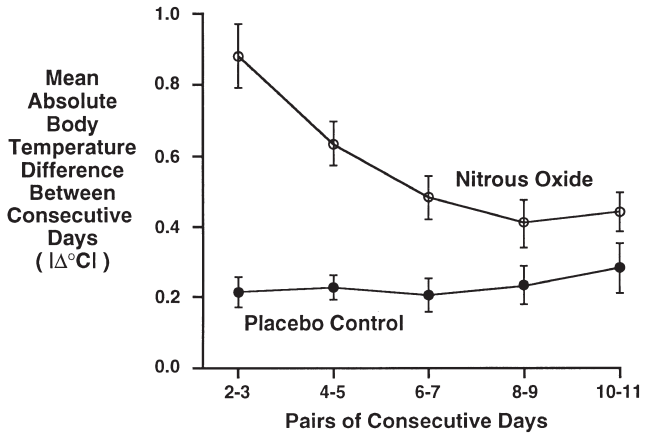

FIG. 3. Absolute temperature difference between consectuive days and standard error bars. Chronic tolerance develops to N_2O's hypothermic effects. Experimental rats (open circles, $n = 32$) displayed a decreasing temperature difference between N_2O and placebo exposures. Control rats (closed circles, $n = 8$) displayed little change in absolute temperature difference between successive days.

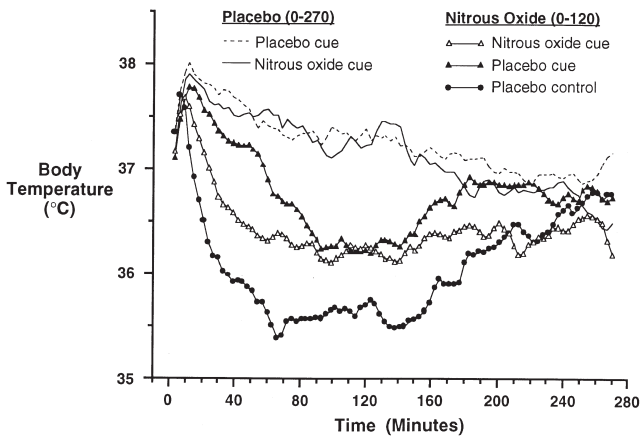

FIG. 5. Mean body temperature on day 12. There was no evidence that providing the drug cue independently from the drug being delivered influenced body temperature. Rats in the N_2O groups breathed N_2O from 0–120 min followed by placebo gas from 120–270 min. Rats in the placebo groups breathed the placebo gas during the entire exposure (0–270 min). The average standard error of the mean for the five groups was 0.21 C (range, 0.15–0.27°C).

($n = 4$) received a 380-min exposure to 60% N_2O and 40% O_2 on the first day, and then on the following day received a 380-min exposure to placebo gas. The other squad ($n = 4$) was treated identically, except that the order of the N_2O and placebo exposures was reversed.

Data analysis. Acute tolerance was assessed by fitting nonlinear mixed-effects models by the restricted maximum likelihood method (22) to the temperature differences for each rat (N_2O session minus placebo session). A piece-wise linear regression model was assumed in which the temperature difference for each rat followed a linear model up to a change point, where the slope of the temperature difference curve was allowed to change. The change point is the time at which the temperature stopped decreasing and began increasing during N_2O exposure. The model allows all parameters, including the initial temperature and slope, the slope after the change point, and the time of the change point itself, to vary from rat to rat with a normal distribution. No adjustment for squad effects was used because of the existence of the randomly varying parameters and the potential problems with over parameterizing the model.

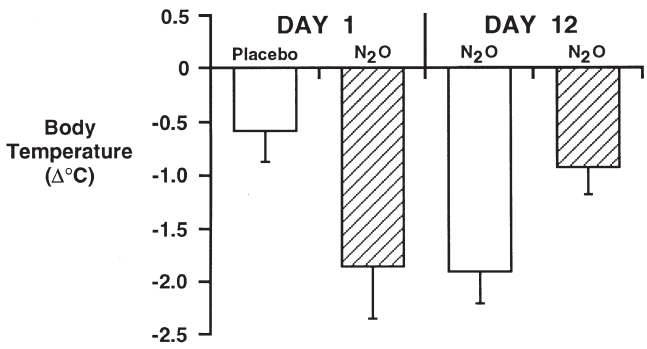

FIG. 4. Mean maximum reduction of body temperature from baseline between 0 and 120 min and standard error bars. The open bars represent the placebo control condition, while the shaded bars represent the subset of experimental rats whose N_2O and odor pairings were the same on days 1 and 12.

Results

Rats initially became hypothermic in the presence of N_2O, as demonstrated by a negative slope in the initial portion of the temperature difference curve (mean slope = −0.016°C per minute, SEM = 0.004°C, $p < 0.001$). The decrease of temperature was followed by a statistically significant positive slope after the change point, thus providing statistical evidence for acute tolerance (mean slope = 0.0038°C per minute, SEM = 0.0007, $p < 0.001$). The between-rat standard deviation of the postchange point slopes was 0.0018°C per minute, suggesting that the majority of rats would exhibit acute tolerance, and only a small percentage of rats would exhibit very little or no acute tolerance over a 380-min exposure. The average change point occurred at 141 min, with a standard deviation of 30 min (thus, roughly 95% of the change points would occur between 80 and 200 min after the start of exposure). Finally, there was an estimated negative correlation ($r = -0.72$) between the rate of acute tolerance development (temperature slope after the change point) and the time of the change point, indicating that rats exhibiting a faster rate of intrasessional recovery experienced the temperature upturn (relative to the placebo session) sooner. Figure 6 displays the observed temperature difference data for the individual rats and the average of the piece-wise linear fitted curves.

Discussion

Increasing the duration of N_2O exposure permitted enough animals to develop acute tolerance so that the phenomenon could be described statistically. A common caveat that is mentioned in many experiments that do not find evidence for acute tolerance development is that the duration of the drug exposure may have been too short to observe the phenomenon. This is important because, as Ramsay and Woods (31) have suggested, if chronic tolerance develops to a

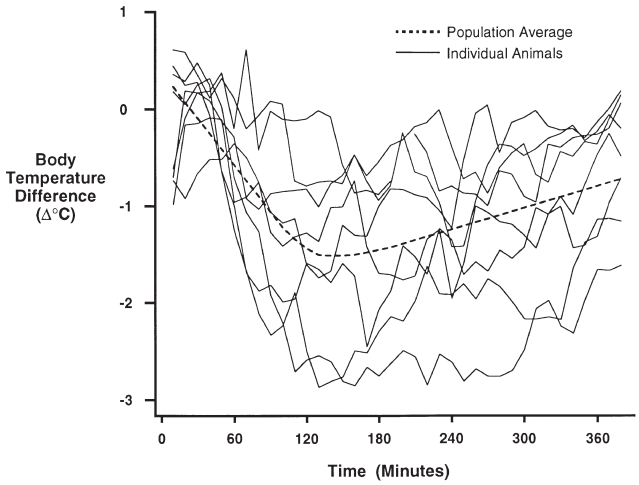

FIG. 6. Acute hypothermic tolerance develops during a 380-min exposure to 60% N_2O. Data are presented from the individual rats as well as the average piece-wise linear fitted curve illustrating acute tolerance development.

particular drug effect, then acute tolerance should be documentable on the first exposure if the duration of the exposure is sufficient.

GENERAL DISCUSSION

Several important conclusions result from these experiments. First, 60% N_2O causes a robust hypothermic effect in rats, supporting the findings of Quock and colleagues (28). Second, chronic tolerance develops to this hypothermic effect. Within-subject comparisons clearly illustrate the development of chronic tolerance over drug exposure sessions (cf., Fig. 2). In addition, N_2O hypothermia exhibited by a separate group of control subjects rules out any interpretation that chronic tolerance might have developed as an artifact of the experimental procedures per se (cf., Fig. 4). Third, approximately 95% of rats begin to recover from N_2O's hypothermic effects (i.e., exhibit acute tolerance) between 80 and 200 min of continuous exposure to a constant concentration of 60% N2O. Thus, the present studies demonstrate that both acute and chronic tolerance develop to N_2O's hypothermic effects.

Understanding drug tolerance is important for both practical and theoretical reasons. At the practical level, tolerance is known to develop within a variety of drug classes and can pose a significant problem to clinicians in the treatment and management of patients. Theoretically, tolerance is of interest to scientists studying the processes underlying drug addiction (15). For example, tolerance is often associated with the development of physical dependence such that tolerance development increases in tandem with the intensity of drug withdrawal. Consequently, numerous investigators [e.g., (13,16, 18,27,31)] have suggested that tolerance and dependence result from a common underlying mechanism. Interestingly, withdrawal-like rebound effects have been observed following the discontinuation of N_2O in subjects that have developed tolerance (9,33). Experiment 1 included a 150-min assessment period following the discontinuation of a 120-min exposure to 60% N_2O in an attempt to determine whether a hyperthermic rebound effect might develop. By the end of the 150-min recovery period, the values of the control and experimental groups had merged (see Fig. 1), but there was no way to determine whether a hyperthermic rebound effect would have developed had the data collection period been extended. However, it is also the case that there was no evidence for acute tolerance development during this initial exposure, and thus, a rebound effect may not have been anticipated.

Another method for studying withdrawal-like rebound effects is through the process of Pavlovian conditioning (39). Following a Pavlovian conditioning paradigm, chronically tolerant animals that are given a drug predictive cue but that are administered a placebo drug often exhibit a conditioned response that resembles a withdrawal-like effect (38). In addition, chronically tolerant animals given a placebo predictive cue but that receive the active drug do not appear tolerant (i.e., they exhibit environmentally specific tolerance) (40). In Experiment 1, we attempted to classically condition the animals by pairing the administration of N_2O and placebo each with its own unique odor cue (almond or banana). However, no evidence of classically conditioned thermic changes were observed on the test day, although chronic tolerance had clearly developed. There are several possible reasons why classical conditioning was not observed. For example, the same gas exposure chambers were used for all trials regardless of whether placebo or N_2O was administered. The only difference was the odor cue. It may be that a single odor cue was not sufficient for the animals to learn the discrimination. Alternatively, additional conditioning trials may have been needed for the animals to learn the discrimination. Yet another possibility is that the initial interoceptive cues of N_2O serve as a potent cue that signals the upcoming drug effects (11). Finally, of course, conditioning processes may not contribute to the development of chronic tolerance to N_2O's hypothermic effect. Future work on conditioning N_2O's thermic effects should consider using maximally distinguishable drug exposure environments, a sufficient number of drug exposure trials to develop maximal tolerance, and an additional group that receives a brief "priming" dose of N_2O on the conditioning test day to provide an interoceptive drug cue (12).

Large individual differences were observed with respect to N_2O's hypothermic effect. This aspect of the data is not obvious from an examination of the averaged temperature data presented in Fig. 1. In the first experiment, 36 N_2O-naive rats received a 2-h exposure to 60% N_2O. Some individual rats appeared initially insensitive to N_2O hypothermia, while others had a large hypothermic effect that continued to decline throughout the N_2O administration, and still others demonstrated a clear recovery of body temperature (i.e., acute tolerance) despite the continuous administration of N_2O. Interestingly, large individual differences are often noted in human research with N_2O (2,7,8,17,29,30).

In the alcohol literature (21,44,45,35,36), numerous investigators have suggested that reduced initial sensitivity as well as heightened acute tolerance development may be related to an individual's increased vulnerability to subsequent drug abuse and addiction. Ramsay and Woods (31) have suggested that acute tolerance (and possibly initial insensitivity) may reflect a heightened responsiveness on the part of some individuals to a drug induced perturbation, which in turn, makes those individuals more vulnerable to the psychophysiological changes underlying addiction. The pharmacological properties of N_2O are well suited to investigate intrasessional factors like initial sensitivity and acute tolerance. Future research should investigate the relationship between these intrasessional factors during an initial drug exposure and the subsequent development of chronic tolerance.

ACKNOWLEDGEMENTS

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