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Reliability of individual differences in initial sensitivity and acute tolerance to nitrous oxide hypothermia

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Abstract

On average, the hypothermia exhibited by rats receiving 60% nitrous oxide (N_2O) eventually abates despite the continued inhalation of the drug (i.e., acute tolerance develops). However, large individual differences occur in both the magnitude of hypothermia achieved and the degree of acute tolerance that develops. To determine whether the degree of temperature loss and subsequent recovery during N_2O administration are reliable characteristics of an individual, we measured intraperitoneal temperature via telemetry in 77 Long–Evans rats that each received 60% N_2O for 5 h during two sessions separated by 14 days. Good intersession reliability (Pearson's r) was observed for simple change and adjusted change scores for both initial N_2O temperature sensitivity ($.61 \leq r \leq .62$), and acute tolerance development ($.46 \leq r \leq .52$). In a separate experiment, three groups of rats were selected based on their individual body temperature patterns during an initial N_2O administration: (1) insensitive to N_2O hypothermia ($n = 8$); (2) marked hypothermia followed by acute tolerance development ($n = 6$); and (3) marked hypothermia followed by little acute tolerance development ($n = 6$). When retested 10 days later, each group exhibited a body temperature profile similar to that observed during the initial N_2O exposure. Thus, the temperature profile observed during a rat's initial exposure to 60% N_2O reflects a reproducible response for that animal.

Keywords: Addictive vulnerability; Acute tolerance; Chronic tolerance; Addiction; Drug sensitivity; Reliability

1. Introduction

Drug tolerance, the reduction of a drug's efficacy over time, not only favors an escalation of drug consumption, but may derive from mechanisms that also contribute to dependence, addiction, and the severity of withdrawal symptoms (Kalant, 1996; Kalant et al., 1971; Poulos and Cappell, 1991; Ramsay and Woods, 1997). Drug tolerance develops over repeated drug administration sessions (chronic tolerance), and can also develop within an initial drug administration session (acute tolerance). Pre-existing individual differences in acute tolerance may predict subsequent levels of chronic tolerance, and thus play an important role in addictive vulnerability (Beirness and Vogel-Sprott, 1984; Kalant et al., 1971; Li et al., 1993; Ramsay and Woods, 1997; Wilson and Plomin, 1985). Initial drug sensitivity also varies among individuals, and low initial sensitivity may increase the risk of drug addiction (Schuckit, 1994).

Fundamental assumptions of these hypotheses are that levels of initial drug sensitivity and acute tolerance are reliable characteristics of an individual. The reliabilities of various measures of sensitivity and acute tolerance have been examined in human subjects (Nagoshi and Wilson, 1989; Wilson and Nagoshi, 1987). However, such data are lacking in the animal literature. Thus, a goal of the present study was to develop a rigorous animal model to determine the reliability of individual differences in sensitivity and acute tolerance to drug-induced hypothermia.

The use of the pharmacologically active gas nitrous oxide (N_2O) has advantages for studying the relationships among initial drug sensitivity, acute tolerance, and chronic tolerance. In addition to its euphorigenic, analgesic, and anesthetic effects (Russel et al., 1990; Smith and Wollman,

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1985), subanesthetic concentrations of N_2O cause hypothermia in a dose-related manner (Pertwee et al., 1990; Quock et al., 1987; Ramsay et al., 1999), which can be measured continuously and without stress via an implantable telemetric temperature sensor. Rats develop both acute and chronic tolerance to N_2O's hypothermic effect, and the temperature profiles observed during an initial N_2O administration display wide individual variability (Ramsay et al., 1999). Furthermore, there is face validity for studying N_2O in a context of addiction-related phenomena owing to its 200-year history as a drug of abuse (Layzer, 1985) and because nonhuman primates will self-administer it as well (Wood et al., 1977).

Because changes in drug concentration can alter behavioral and physiological measures during a drug administration session, acute tolerance is best studied at a steady-state drug concentration (O'Connor et al., 1998; Ramsay and Woods, 1997; Ramsay et al., 1999). By administering a constant concentration of N_2O, a steady-state concentration is rapidly established and maintained in the central nervous system (Eger, 1985), thereby facilitating the measurement of acute tolerance. N_2O undergoes almost no metabolism in the body (Trudell, 1985), thus precluding pharmacokinetic (dispositional) explanations for the development of acute and chronic tolerance.

In an initial experiment, we evaluated the reliability of individual differences to N_2O hypothermia by comparing body temperature decreases and subsequent temperature recovery values in rats measured during two 5-h exposures to 60% N_2O that were separated by 14 days. In a second experiment, groups of rats selected for initial N_2O-associated temperature profiles that exemplify one of three patterns (insensitive, sensitive with acute tolerance, and sensitive without acute tolerance) were retested 10 days later to examine whether such groups would retain their distinct temperature profiles.

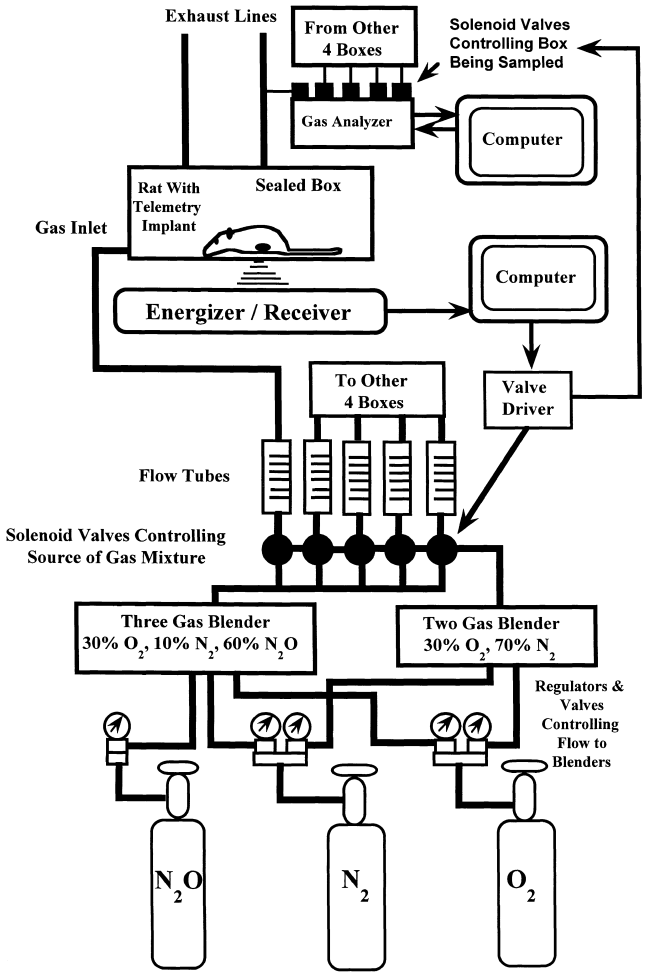

Fig. 1. Schematic of the computer-controlled gas delivery system used to investigate the reliability of N_2O-induced hypothermia. Gases flow to gas blenders and then to five solenoid valves, one for each of five exposure chambers, to deliver 60% N_2O or placebo gas into each chamber. Solenoids are actuated by a computer-controlled valve driver. Each rat is placed in a 10-l exposure chamber. A platform below energizes the implanted temperature sensor. Temperature data are acquired and stored by computer. Gas is sampled from each chamber for gas concentration analysis via a second computer.

2. Methods

2.1. Gas delivery and data acquisition system

A custom-built, automated system controlled gas delivery and data acquisition in both Experiments I and II (Fig. 1). Medical grade oxygen (O_2), nitrogen (N_2), and N_2O were delivered to regulator assemblies that provided gas flow to two gas blenders. All three gases were delivered at 110 psi to a proportional gas blender (Model 299-037F, Smith Welding Supply and Equipment, Detroit, MI), which was adjusted to produce an outflow consisting of 30% O_2, 10% N_2, and 60% N_2O (referred to as 60% N_2O). Additionally, N_2 and O_2 were delivered at 60 psi to a two-gas blender (Bird, Palm Springs, CA), which was adjusted to produce an outflow consisting of 30% O_2, 70% N_2 (referred to as placebo gas). Gas from each blender flowed to five computer-controlled solenoid valves (Parker Hannifin, Fairfield, NJ, product number 004-0007-900), which determined whether 60% N_2O or placebo gas was delivered to each of the five exposure chambers described below. The solenoids were actuated by a valve driver (Parker Hannifin, product number 090-0032-100-1) controlled by VitalView software (Mini-Mitter, Sunriver, OR) operating on a personal computer. Gas from each solenoid valve passed through a flow tube (Dwyer Instruments, Michigan City IND, model VFA-24-BV), adjusted to produce a flow rate of 3.0 l/min, and then through an inlet into the exposure chamber. Two lines affixed to outlets in each chamber's lid provided a low-resistance path by which exhaust gas flowed to a fume hood. During all studies, effluent gas was sampled every 3 min for a 30-s interval from each chamber and analyzed for fractional composition of N_2O, O_2, and CO_2 by an infrared gas analyzer (Normocapoxy, Datex Instruments, Helsinki, Fin-

land). Serial sampling of effluent gas and room air (a control) used a second set of five solenoid valves actuated by the valve driver. Gas sampling and data acquisition were controlled by a program written in LabView 5.0 (National Instruments, Austin, TX) installed on a Macintosh IIci computer (Apple Computers, Cupertino, CA).

Each exposure chamber (Columbus Instruments, Columbus, OH) was made of clear Lexan, and had a removable lid secured with four latches and sealed with a 1/4-in. closed cell silicone foam rubber gasket. During trials, the floor of the chamber was covered with a layer of wood shavings. The chamber's internal dimensions were $18 \times 18 \times 31$ cm, yielding a volume of 10 l. Given this volume and a gas inflow (and outflow) rate of 3 l/min, the theoretical half-time by which the chamber achieves the target gas composition is 2.3 min (Lewis et al., 1987). The chamber's gas environment is thus calculated to reach 97% of the administered gas concentration in 11.6 min. This figure was confirmed by the infrared gas analyses of N_2O and O_2. The steady-state gas concentrations (mean ± standard deviation) averaged across the two experiments reported in this paper were as follows: N_2O, 59.8 ± 0.5%; O_2, 29.6 ± 0.5% (60% N_2O mixture); O_2, 29.6 ± 0.5% (placebo mixture). Levels of CO_2 averaged 0.2 ± 0.01%. Each exposure chamber was placed on a platform that both energized the implanted temperature sensor, and received its temperature signal output. Temperature data were acquired using VitalView software (Mini-Mitter). The five energizer/receiver platforms were positioned side-by-side on the second tier of a four-tiered plastic shelf assembly. During all studies, an opaque black vinyl drape was suspended in front of the exposure chambers, and panels made of the same material were hung between the chambers to visually isolate each chamber.

2.2. Experiment I

2.2.1. Animals

A total of 120 male Long–Evans rats weighing 200–250 g were obtained from a breeding colony maintained by the University of Washington Department of Psychology. Data from 94 of these rats (77 experimental and 17 control rats) are reported in this paper; data from 24 animals were incomplete because their implanted epoxy-coated temperature sensor (E-mitter Model PDT4000G, Mini-Mitter) did not provide usable temperature data in the second exposure session. [Sensor failure was due to moisture leakage (personal communication with manufacturer). This sensor was superseded by the leak-proof glass-enclosed model used in Experiment II.] One rat's data were excluded because in the second drug administration session, a baseline intraperitoneal temperature of 34°C (5.0 standard deviations below the mean) was recorded. The breeding colony in the Psychology Department is supplemented at least annually with breeding animals (Simonsen Laboratories, Gilroy, CA) and no sibling matings are permitted. Rats were housed in an AAALAC accredited facility in individual stainless steel hanging cages with laboratory chow and water available ad libitum, and maintained on a 12:12 h light/dark cycle (0700–1900 h) at an ambient temperature of 22 ± 1°C, while the experiments described below were conducted at an ambient temperature of 20 ± 0.5°C. Animals were not fasted prior to the experiments involving N_2O or placebo gas administration. This research was approved by the Institutional Animal Care and Use Committee, Department of Comparative Medicine, University of Washington.

2.2.2. Surgical methods

Each rat was anesthetized by an intraperitoneal injection of 0.5 ml/kg of a solution containing 90.9 mg/ml ketamine and 1.8 mg/ml xylazine (Phoenix Pharmaceuticals, St. Joseph, MO), and a temperature sensor was implanted into the peritoneal cavity.

2.2.3. Protocol

At least 7 days following surgical sensor implantation, every rat received a single 1-h habituation trial during which placebo gas was administered. Exactly 1 week after the habituation trial, each rat received the first of two 6-h gas exposure sessions. These trials commenced at 0945–1015 h. During the first hour, all rats received placebo gas (baseline period). During the next 5 h (treatment period), the rats in all but the middle chamber received the N_2O mixture, while the rat in the middle chamber continued to receive placebo gas. A 5-h N_2O administration period was chosen based on previous research indicating that acute tolerance to the hypothermic effect would develop within that period (Ramsay et al., 1999). Exactly 14 days later, each rat was retested in the same exposure chamber and location.

2.2.4. Data analysis

2.2.4.1. Modeling of individual temperature data. For each rat, intraperitoneal temperature values were recorded at 30-s intervals throughout each 6-h study. The median temperature values of successive 15-min intervals were used to represent the body temperature profile of each animal. A piece-wise least-squares linear regression model (S+, Mathsoft, Seattle, WA) was fit to each rat's median temperature values during the 5-h treatment period. This model represents the temperature profile as two straight lines, one for the initial temperature decrease to a change point (point where temperature slope reverses), the other for the recovery of temperature toward baseline (Fig. 2). The model allows all parameters, including the initial temperature and slope, the time of the change point, and the slope after the change point, to vary from rat to rat.

2.2.4.2. Quantification of initial drug sensitivity and acute tolerance. Drug sensitivity and acute tolerance can each be expressed as a simple change score, a common practice dating to 1919 (Mellanby, 1919). A change score is the difference in a dependent variable's value on two occasions

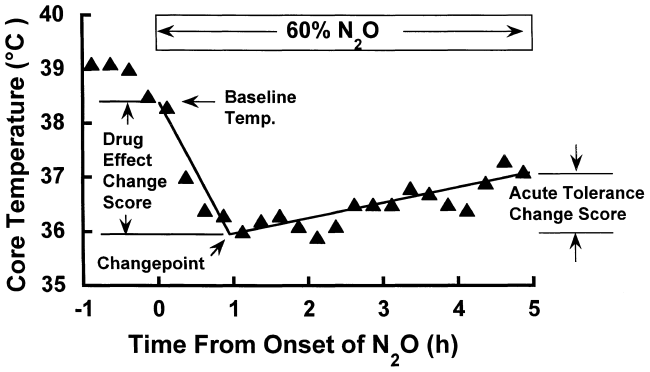

Fig. 2. Representative intraperitoneal temperature profile from one rat exposed to N_2O. Each point is the median of 30 values obtained within a 15-min interval. The two lines were generated by piece-wise linear regression modeling of the temperature data. Illustrations of baseline temperature, change scores, and change point are shown.

during a drug administration session (e.g., lowest body temperature during N_2O administration minus baseline body temperature). However, change scores tend to be correlated with baseline scores because both entities have in common the baseline value. Therefore, a change score can be reliable merely because the baseline score is reliable (Nagoshi and Wilson, 1989; Nagoshi et al., 1986; Rogosa et al., 1982). Additionally, when animals are administered hypothermic drugs such as N_2O, baseline body temperature and change of body temperature might also be correlated because the rate of heat loss is proportional to the difference between the temperature of the animal and the environment (Gordon, 1990; Kleiber, 1972). Hence, change scores that are adjusted for, and therefore uncorrelated with, baseline values (regression residuals) have been recommended for quantifying individual differences in initial sensitivity and acute tolerance to drug administration (Nagoshi et al., 1986).

2.2.4.3. Drug effect scores. The drug effect change score was computed as the difference between the modeled temperature at the change point and the initial, modeled baseline temperature 7.5 min prior to the treatment period. The adjusted drug effect change score was computed as the residual (illustrated in Fig. 5) from the linear model obtained by regressing the drug effect change score on baseline temperature (Nagoshi et al., 1986); i.e., as the error term e_i in the equation.

$$\text{Drug effect change score}_i = (\beta_o + \beta_1 T_{o_i}) + e_i$$

where T_{o_i} is the individual's baseline temperature and β_o and β_1 are the intercept and slope, respectively, of the least-squares linear regression equation that estimates the component of the change score that can be explained by T_{o_i}. Values of the "error" term, e_i, (the regression residual) are uncorrelated with T_o, so e_i represents the component of the change score that reflects something unique about the individual's response and/or measurement error. If this component of uniqueness arises solely from random measurement error, then replicate estimates of e_i will not be correlated. By contrast, if true individual differences exist in the hypothermic effect of N_2O, then replicate values of e_i will be correlated.

2.2.4.4. Acute tolerance scores. The acute tolerance change score was computed as the difference between the modeled temperature at the end of the treatment period and that at the change point. The adjusted acute tolerance change score was computed as the residual from the linear model obtained by regressing the acute tolerance change score on both the initial baseline temperature and the drug effect change score (Nagoshi et al., 1986).

2.2.5. Statistical analyses

Comparisons of mean differences were done using the appropriate t test (within- or between-subjects). Associations between variables were analyzed via linear, or multiple linear, regression. Statistical computing was done with S+ (MathSoft) and SPSS 8.0 (SPSS, Chicago, IL). Significance was set at α=.05, two-tail.

2.3. Experiment II

Three groups of rats formed on the basis of individual differences in N_2O hypothermia received a series of placebo gas exposures and then received a second N_2O exposure. This work therefore permitted an examination of the reproducibility of three distinct patterns of N_2O hypothermia.

2.3.1. Animals

Source, rat strain, initial body weight, housing and surgical procedures were the same as in Experiment I.

2.3.2. Protocol

Rats, in squads of 40, were implanted with the leak-proof temperature sensor (Glass-enclosed E-mitter Model PDT4000G, Mini-Mitter) that superseded the model used in Experiment I. Ten to seventeen days after surgery, each rat received at 0945–1015 h the 1-h placebo gas/5-h 60% N_2O procedure described in Experiment I. After each squad was screened, 10 animals (25%) were identified for retention in the pilot study based on their initial temperature profile during N_2O administration. We retained animals that exhibited minimal hypothermia while receiving N_2O (designated as insensitive), animals that exhibited a large hypothermic effect that persisted until the end of the treatment period (designated sensitive without acute tolerance), and animals that manifested a large hypothermic effect followed by a robust return of temperature toward baseline (designated sensitive with acute tolerance). Extreme standardized values of the adjusted drug effect change score and adjusted acute tolerance change score (see Experiment I) were used to identify the exemplars of the three categories. The animals were given an every-other-day series of five

exposures to placebo gas. Between 14 and 24 days following their initial N_2O exposure, rats were then restudied for temperature responses during a second 1-h placebo gas/5-h 60% N_2O treatment period. The groups were comprised of eight animals designated as insensitive, six as sensitive without acute tolerance, and six as sensitive with acute tolerance.

2.3.3. Data analysis

Intraperitoneal temperature values were recorded at 30-s intervals, and all values obtained during each 5-h N_2O exposure session were used in the calculation of the means shown in Fig. 7. A drug effect change score was computed as the minimum intraperitoneal temperature achieved during the 5-h treatment period minus the temperature at N_2O onset. The significance of group differences was not evaluated because the groups were initially selected to be different. Chronic tolerance development was evaluated using the paired *t* test.

3. Results

3.1. Experiment I

3.1.1. Temperature profiles

Mean intraperitoneal baseline temperatures 7.5 min before commencing the experimental period did not differ between the placebo and N_2O groups ($P=.9$) or between the first and second exposure sessions ($P=.50$); the average over groups and sessions was 37.3 ± 0.06°C. During the experimental period, hypothermia occurred in the N_2O group in both exposure sessions (Fig. 3). The difference between groups (N_2O minus placebo) at 1.1 h

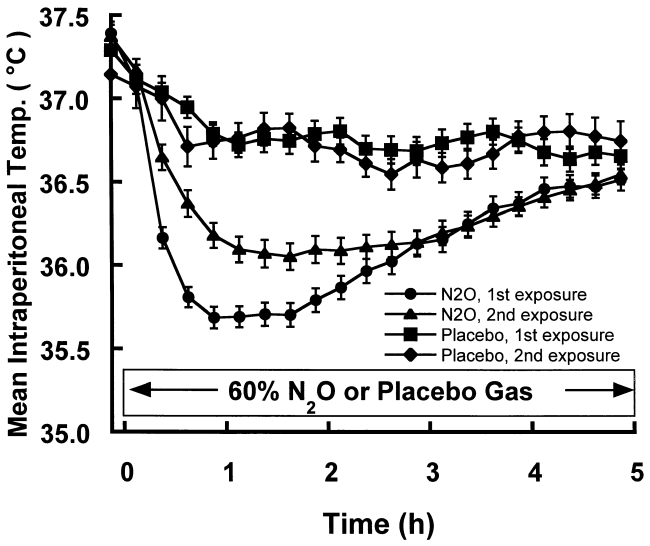

Fig. 3. Mean intraperitoneal temperatures with standard error bars during N_2O and placebo gas administration. Note development of acute tolerance and the blunted hypothermic effect on the second session in N_2O group indicative of chronic tolerance development.

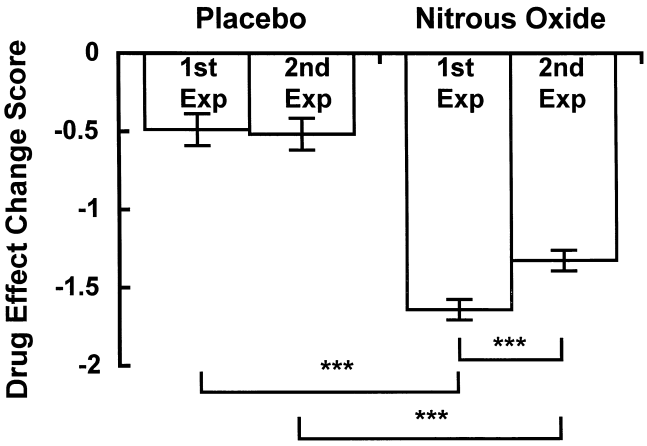

Fig. 4. Drug effect change scores by group and exposure session (Exp). Differences labeled with asterisks are significant at $P<.0001$.

was -1.0°C (first session) and -0.7°C (second) ($P<.0001$ for both sessions). Note that the hypothermic effect of N_2O was attenuated in the second exposure session ($P<.0001$), indicative of chronic tolerance development (Fig. 3). The placebo group's decrease of intraperitoneal temperature in the second exposure session (-0.52 ± 0.10°C) did not significantly differ ($P=.8$) from the first session (-0.49 ± 0.08°C) (Fig. 4). Acute tolerance development, judged by comparing the temperature at the end of the session with temperature at the change point, occurred in the N_2O group in both Exposure 1 (mean increase, 1.1°C; $P<.0001$) and Exposure 2 (mean increase, 0.64°C; $P<.0001$). Indeed, at the end of the 5-h experimental period, the temperature of the N_2O group differed from the placebo group by only -0.35°C during the first exposure ($P=.02$) and by -0.13°C during the second ($P>.05$).

3.1.2. Drug effect scores

In both exposure sessions, the drug effect change score was significantly lower ($P<.0001$) during N_2O than during placebo gas administration (Fig. 4). In the first session, the drug effect change score was -1.64 ± 0.07°C (N_2O group) vs. -0.49 ± 0.08°C (Placebo group). In the second exposure session, the respective values were -1.32 ± 0.08°C vs. -0.52 ± 0.10°C. Therefore, significant chronic tolerance developed to N_2O hypothermia measured in terms of the drug effect change score ($P<.0001$) (Fig. 4).

3.1.3. Reliability of drug effect scores

In the N_2O group, the test–retest Pearson's *r* value (reliability) for the drug effect change score was significant ($r=.61$, $P<.0001$; Fig. 5).

Within each exposure session, the drug effect change score was significantly associated with the baseline intraperitoneal temperature 7.5 min before starting drug administration (Fig. 5). The baseline temperature was reliable ($r=.66$; $P<.0001$) across the two sessions. After adjustment

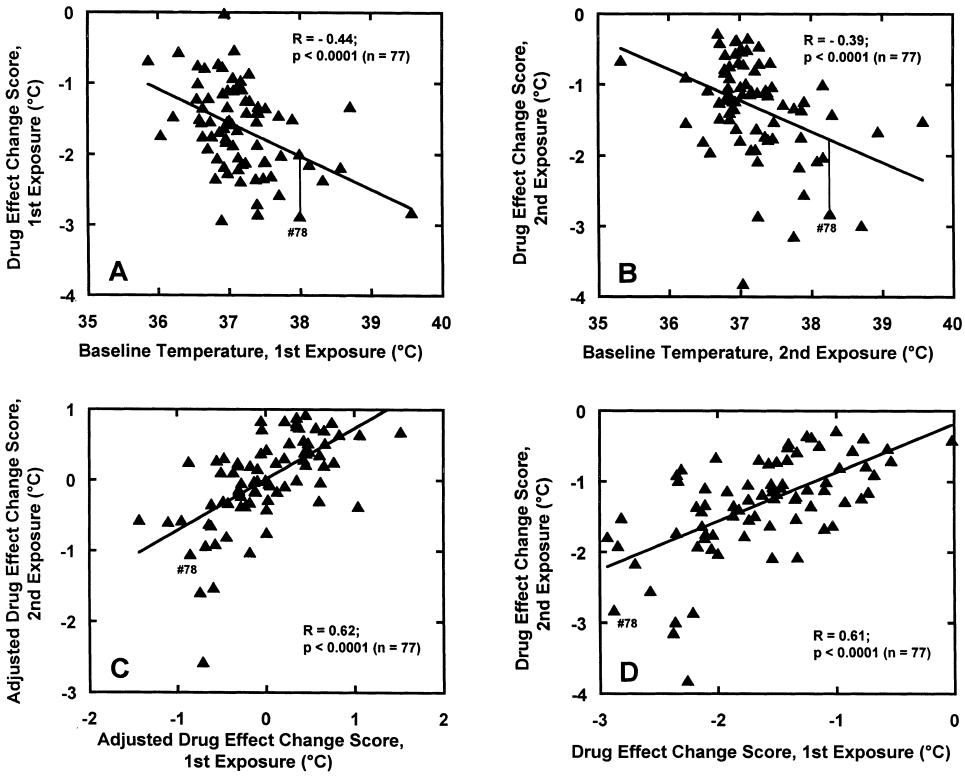

Fig. 5. Panels A and B depict inverse association between drug effect change score and baseline temperature in the two N_2O exposure sessions. The line projecting down from regression fit is the regression residual representing the adjusted drug effect change score for rat # 78 (see Methods). Note similarity of this measure on both exposure sessions. Panel C depicts reliability of adjusted drug effect change scores, and Panel D that of simple drug effect change scores.

for baseline temperature, the test–retest reliability using the adjusted drug effect change score (correlation between regression residuals) was r=.62 (Fig. 5).

By contrast, in the placebo-treated group, no test–retest correlation coefficient was significant for any drug effect score (highest Pearson's r was for drug effect change score, r=.34; P=.17).

3.1.4. Acute tolerance scores

In the N_2O group, the acute tolerance change score was 1.1°C (first session) and 0.64°C (second session). The corresponding values for the placebo group were −0.02°C and 0.02°C. Group differences were significant at P<.0001.

3.1.5. Reliability of acute tolerance scores

In the N_2O group, the test–retest Pearson's r value (reliability) for the acute tolerance change score was r=.53 (P<.0001). The adjusted acute tolerance change score exhibited a reliability of r=.46.

In the placebo group, none of the test–retest correlation coefficients for acute tolerance scores approached significance.

3.1.6. Reliability of actual temperature values

Test–retest correlation coefficients were computed for each of the 15-min median temperature values obtained in the two N_2O exposure sessions (Fig. 6). The majority of r values are between $.5 \leq r \leq .6$. Thus, the reproducibility of the actual 15-min temperature values approximated that seen for the mathematically modeled values for drug effect and acute tolerance as described above.

3.2. Experiment II

The initial N_2O screening procedure yielded three groups of animals that exhibited visually distinct temperature pro-

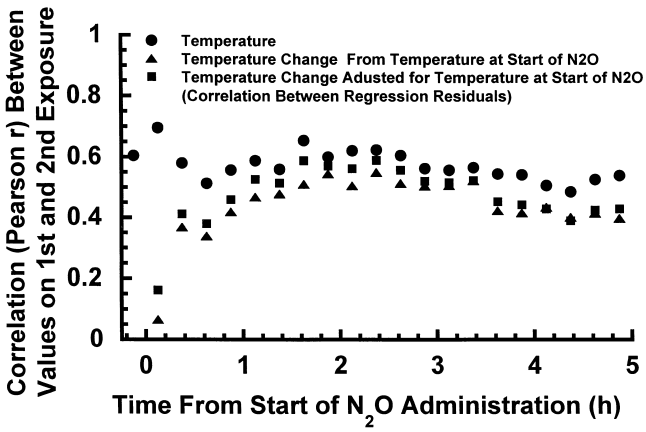

Fig. 6. Correlation coefficients between median intraperitoneal temperature values obtained within each 15-min interval across time.

files during N_2O exposure (Fig. 7). Profiles initially categorized as insensitive (Fig. 7A), sensitive with acute tolerance (Fig. 7B), and sensitive without acute tolerance (Fig. 7C) remained visually distinct in the second session, except for apparent chronic tolerance development as in Experiment I. In the first exposure session, the average drug effect change scores were -0.93 ± 0.29°C (insensitive), -2.38 ± 0.54°C (sensitive with acute tolerance), and -2.78 ± 0.73°C (sensitive without acute tolerance); in the second exposure session, the respective values were -0.39 ± 0.26°C, -1.08 ± 0.56°C, and -1.57 ± 0.57°C. The average acute tolerance change scores in the first exposure session were 0.6 ± 0.12°C (insensitive), 1.9 ± 0.15°C (sensitive with acute tolerance), and 0.78 ± 0.10°C (sensitive without acute tolerance); in the second exposure session, the respective values were 0.61 ± 0.16°C, 1.25 ± 0.25°C, and 0.83 ± 0.08°C. Among all 20 rats, the lowest body temperature during the second N_2O exposure was an average of 0.97°C higher than during the first ($P < .0001$) indicative of chronic tolerance development. The temperature increment was significant within both the insensitive group (mean difference 0.54 ± 0.44°C; $P = .01$) and the sensitive with acute tolerance group (1.3 ± 0.41°C; $P = .02$), and approached significance for the group categorized as sensitive without acute tolerance (1.22 ± 0.49°C; $P = .06$).

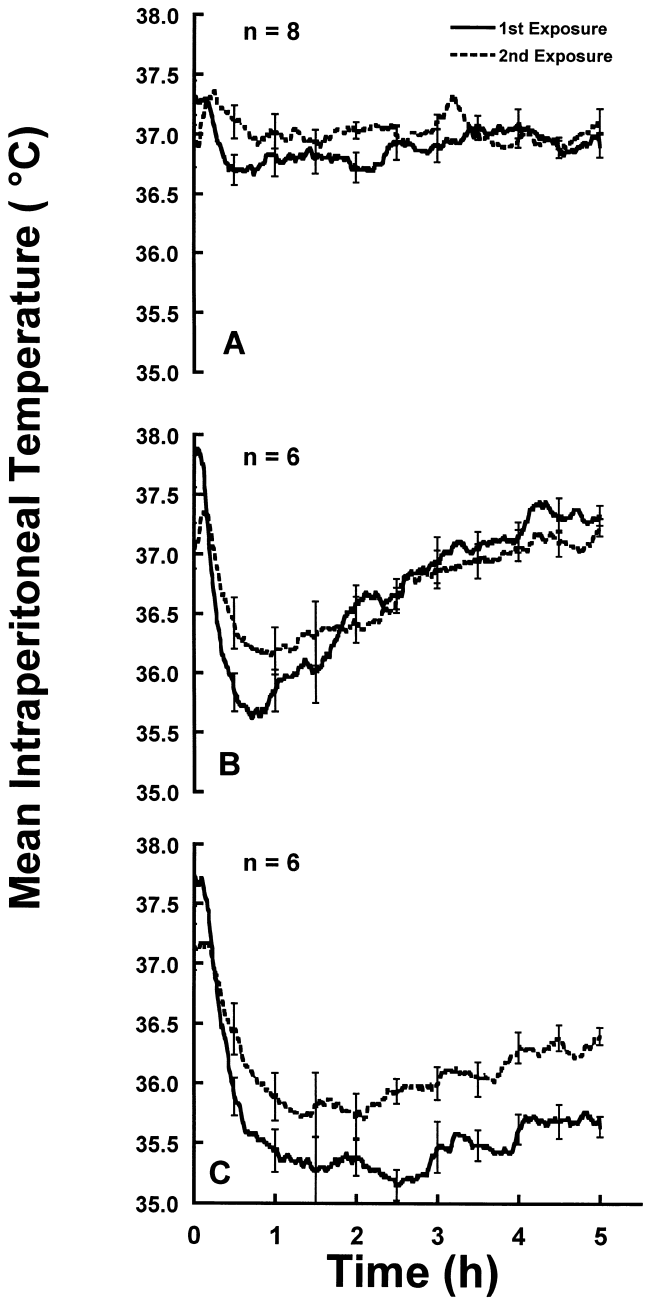

Fig. 7. Core temperature during 5-h exposures to 60% N_2O of rats selected for initial N_2O-associate profiles that exemplify one of three patterns: insensitive (A), sensitive with acute tolerance (B), and sensitive without acute tolerance (C) to N_2O hypothermia. Standard error bars are shown at half-hour intervals to preserve clarity.

4. Discussion

Pre-existing individual differences in initial drug sensitivity and acute tolerance are implicated in the development of chronic tolerance and addictive vulnerability (Beirness and Vogel-Sprott, 1984; Kalant et al., 1971; Ramsay and Woods, 1997; Schuckit, 1994; Wilson and Plomin, 1985). Accordingly, initial drug sensitivity and/or acute tolerance should reflect reliable, idiosyncratic characteristics of the individual. Because limited data bear directly on this issue (Nagoshi and Wilson, 1989; Wilson and Nagoshi, 1987), we developed a rigorous model using the pharmacologically active gas N_2O to assess the reliability of drug-induced hypothermic effects in rats.

In Experiment I, each of the two 5-h exposures to 60% N_2O caused a robust hypothermic effect (Figs. 3 and 4), consistent with previous reports (Pertwee et al., 1990; Quock et al., 1987; Ramsay et al., 1999). In contrast, the placebo group exhibited a small (0.5°C) body temperature decrease in both exposure sessions (Figs. 3 and 4). This decrease could reflect abatement of a residual core temperature elevation caused by handling (Gordon, 1990), or might simply reflect increased convective and evaporative heat loss caused by the 3-l/min flow of gas through the rat's environment. In the second exposure session conducted after a delay of 14 days, the hypothermic effect of N_2O was diminished (Figs. 3 and 4), indicating chronic tolerance development. This result was affirmed in Experiment II. Thus, rats develop chronic tolerance to N_2O's hypothermic effect from a single exposure to the drug 2 weeks earlier. Since our experimental paradigm precludes dispositional explanations of tolerance development, rats must become tolerant to N_2O as a result of learning, pharmacodynamic changes, or both.

The intuitive measure of initial sensitivity to N_2O's hypothermic effect, the drug effect change score, had a

test–retest correlation coefficient of r=.61. Because change scores tend to be correlated with baseline scores, a long-known (Lord, 1958, 1963) but not always appreciated (Nagoshi et al., 1986) problem in measuring change, we computed a drug sensitivity score that was uncorrelated with baseline temperatures using the approach advocated by Nagoshi et al. (1986). The reliability of this adjusted drug change score (r=.62) was essentially that of the unadjusted drug effect change score (r=.61), thus excluding the possibility that well-correlated baseline temperature values (r=.66) spuriously inflated the reliability of the N_2O drug effect.

The acute tolerance change score exhibited a test–retest correlation coefficient of r=.52. Statistically removing the effect of both drug effect and baseline temperature from the acute tolerance change score had little effect on the reliability of this construct (r=.46 for adjusted acute tolerance change score). However, the observation that correlation coefficients for acute tolerance change scores were lower than those obtained for the drug effect scores (r values $\sim$.60) suggests that the reliability of acute tolerance development to N_2O-induced hypothermia is less than that for the initial hypothermic drug effect.

In Experiment II, groups of rats were selected for initial N_2O-associated profiles that exemplify one of three patterns (insensitive, sensitive with acute tolerance, and sensitive without acute tolerance to N_2O hypothermia; Fig. 7). During a second N_2O exposure session, the temperature profiles remained similar to the initial profile observed within each individual difference group. This finding confirms that groups of animals formed using the pattern of N_2O temperature effects are unique in terms of initial sensitivity and acute tolerance.

Although several human studies have assessed the reliability of some indices of drug sensitivity and acute tolerance to drug administration (Nagoshi and Wilson, 1989; Wilson and Nagoshi, 1987), we are unaware of previous animal or N_2O studies that have done so. In human studies investigating the reliability of physiological measures of sensitivity and acute tolerance to alcohol, repeatabilities (Pearson's r) have been in the range, $.0 \leq |r| \leq .32$ (Nagoshi and Wilson, 1989; Wilson and Nagoshi, 1987). The relatively high reliabilities reported in the present study (accounting for approximately 25–40% of the total variance observed) likely reflect the rigor with which our method clamps the drug concentration and measures intraperitoneal temperature. On the other hand, the absence of test–retest reliabilities above r=.66 likely reflects the operation of ongoing nondrug influences that interact with drug administration to alter body temperature (Ramsay and Woods, 1997).

In summary, we found that sensitivity to N_2O-induced decreases of temperature and the development of acute tolerance to this effect both exhibit good levels of reliability in rats. This outcome supports the use of this experimental paradigm to investigate whether initially observable individual differences predict individual differences that develop with repeated drug use.

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