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Nitrous Oxide: Sensory, Motor, Associative, and Behavioral Tolerance Effects in Classical Conditioning of the Rabbit Nictitating Membrane Response

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MOON, Y., M. M. GHONEIM AND I. GORMEZANO. *Nitrous oxide: Sensory, motor, associative, and behavioral tolerance effects in classical conditioning of the rabbit nictitating membrane response.* PHARMACOL BIOCHEM BEHAV 47(3) 523-529, 1994.—Experiment 1, of a series of six experiments with the rabbit nictitating membrane response (NMR) preparation, revealed that nitrous oxide (0%, 33%, 67%) impaired acquisition of conditioned responses (CRs). Subsequent experiments indicated that nitrous oxide (N_2O) had no reliable effects upon nonassociative processes (Experiment 2); impaired unconditioned response (UR) amplitude (Experiment 3); attenuated tone-conditioned stimulus (CS) intensity (Experiment 4); decremented tone-induced reflex modification of the unconditioned NMR (Experiment 5); and demonstrated no reliable evidence of behavioral tolerance (Experiment 6). It was concluded that N_2O's impairment of CR acquisition was attributable to its attenuation of the intensity of tone CSs and shock USs and/or UR amplitude. These findings are consistent with the behavioral laws of conditioning: the attenuation of the intensive sensory properties of the CS and US and/or UR components of conditioning affect their ability to enter into the establishment of CS-CR connections and, therefore, the development of associative learning.

Anesthetic Nitrous oxide Classical conditioning Learning Tolerance Rabbit Nictitating membrane response

THE possible occurrence during general anesthesia of perception and retention of information such as operating room conversations is of substantial clinical concern and theoretical interest (3). We do not know the concentrations of anesthetics that prevent learning. This information is necessary because there are many clinical situations where patients can only tolerate "light" anesthesia (e.g., caesarean section, major trauma cases, severe cardiovascular, or other systemic disease), and many anesthesiologists avoid using a high concentration of inhalation or intravenous anesthetics in favor of using low concentrations of anesthetics supplemented with muscle relaxants and perhaps some opioids. Opioids in the dosages used have neither anesthetic nor amnesic effects and there is no measurement that guarantees unconsciousness in the paralyzed patient. We also do not know the mechanisms by which anesthetics affect learning and memory.

We chose the rabbit's nictitating membrane response (NMR) to study the effects of anesthetics. This preparation represents classical or Pavlovian conditioning, which is one basic category of associative learning (16). A neutral tone or *conditioned stimulus* (CS) is paired with an *unconditioned stimulus* (US) consisting of a paraorbital shock. Initially, only the US elicits extension of the nictitating membrane across the cornea, the *unconditioned response* (UR). However, when the CS and US are then presented repeatedly to the rabbit in a specified order and temporal spacing, a response similar to the UR develops to the CS, that is, *conditioned response* (CR) (4).

When studying the effects of drugs on classical conditioning, there are three potential drug-behavior interactions. The drug may impair the ability of the animal to associate the CS and US (i.e., learn), or it may act on conditioning by altering

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the sensory processing of the CS and US, or the drug may impair the motor expression of either the CR or UR. It is also important to control for unlearned or nonassociative processes by using unpaired stimulus procedures.

In the present series of studies, we used nitrous oxide, the most commonly used anesthetic, to determine its effects on acquisition and to localize the drug's effects upon associative, nonassociative, sensory, and motor processes. Because classical conditioning of the NMR of the rabbit needs repeated pairings of the CS and US over several days, it is important to determine whether behavioral tolerance develops to repeated daily exposures to nitrous oxide, and thus attenuates its effects. Specifically, first, we determined the effect of the drug on the acquisition of CRs under CS-US pairings. Second, we sought to determine the contribution to nitrous oxide's impairment of CR acquisition to its effects on the nonassociative processes observed under unpaired CS-alone and US-alone presentations, such as sensitization, pseudoconditioning, baseline responding, and amplitude of the UR. Third, we determined whether nitrous oxide was affecting the sensory processing of the US by measuring the frequency and amplitude of URs elicited by varying US intensities. Fourth, using previously trained animals, we determined whether nitrous oxide affected the conditioned excitatory properties of a tone CS by measuring the percentage of CRs elicited at varying CS intensities. Fifth, we determined whether nitrous oxide altered the unconditioned excitatory properties of a tone stimulus in experimentally naive animals by measuring the ability of a tone to produce facilitation of the NM reflex. Finally, we examined the development of behavioral tolerance due to repeated exposures to nitrous oxide.

METHOD

Animals

A total of 183 male and female New Zealand white albino rabbits were used. The rabbits weighed approximately 2 kg on arrival from Knapp Creek Rabbitry (Amana, IA). The animals were housed individually with free access to food and water. Consistent with their rearing conditions, animals were kept in constant light.

Apparatus and General Procedures

For recording the NMR, a length of 6-0 monofilament nylon was sutured through the epithelium layer of the membrane. The animal was then restrained in a Plexiglas box, a headmount containing a photoresistive transducer was secured on the animal's head, and US leads were applied to the paraorbital region to deliver an electrotactile stimulus (100 ms, 60 Hz, 3 mA). A rod mechanically coupled to the shaft of the transducer was attached to the loop in the nictitating membrane by a retractable hook. Each rabbit inside a Plexiglas restrainer was positioned into one of eight ventilated and sound-attenuated chambers for NMR or training. Each chamber contained a speaker for presenting a 400-ms, 84-dB, and 1-kHz tone. An Apple II/FIRST system controlled the presentation and duration of stimuli and conducted analog-to-digital conversion of the NMR signals generated by the transducer (4,6).

Nitrous oxide was administered to each animal independently from an assembly of oxygen and nitrous oxide flowmeters. Gases flowed into the inlet port of a Jackson-Rees modification of Ayre's T-piece. The concentration of nitrous oxide was monitored by a Puritan-Bennett gas analyzer. The anesthesia circuit was attached to a face mask designed with minimum dead space and allowed free movement of the NMR transducer assembly. Specifically, the animal's end of the mask was covered by a rubber membrane with a small hole cut in the center for the rabbit's nose and mouth to snugly enter the mask with a gas-tight fit. The mask was secured to the animal by velcro strips attached to the mask and wrapped around the nape of the rabbit's neck. Corrugated tubing was attached to the end of the modified T-piece and a bag was attached to the other end. The end of the bag was fitted to an exhaust tubing connected to a gas scavenging system. To prevent rebreathing and carbon dioxide retention, we administered fresh gas flow of three times the minute ventilation (we conservatively administered 3 L/min). Nitrous oxide was administered in three doses to separate groups of animals: 0%, 33%, and 67% concentrations in oxygen.

Response Measurement

A response was defined as an NM extension of at least 0.5 mm, and the frequency, amplitude, and onset latency of each response were recorded. Responses occurring in the CS-US interval were classified as CRs, and those occurring within 100 ms after shock-US onset were recorded as URs. For groups receiving unpaired CS, US training (Experiment 2), responses that were recorded during the 800-ms duration of the CSs provided an assessment of nonassociative sensitization (i.e., enhancement of unconditioned responding to the CS resulting from presentations of the US). (This enhancement occurs in the absence of any relationship between the two events. If unconditioned responses to the CS resemble the conditioned response of interest, one might falsely conclude that conditioning had occurred.) Responses were also recorded for pseudoconditioning (i.e., responses appearing in the CR measurement interval, when exposure to the US results in the CS eliciting responses similar to the unconditioned response). (This process occurs in the absence of any pairings of the two events and may result from generalization between the CS and US.) Responses occurring during the 800-ms interval preceding each US presentation provided an assessment of the base rate. Finally, the frequency, onset latency, and peak amplitude of the URs were recorded for each US-alone trial.

Adaptation

All rabbits were given 1 day of adaptation equal in length to their subsequent experimental sessions. No stimuli were presented, only 100% oxygen was supplied, and NMRs were recorded at times corresponding to the subsequent CS-US observation intervals to obtain baseline rates of responding.

Data Collection and Statistical Analyses

We used our Apple/FIRST system (17) for data collection. The system controls also the presentation of stimuli and online digitizing of analog signals of the NM. During intertrial intervals, digitized response topographies are displayed with: a) CR and UR latency; b) CR and UR amplitude; c) latency of the peak amplitude of CRs and URs; and d) area under the CR and UR topographies. After each trial, all response measures are stored on a Corvus 20M hard disk. At the end of an experimental session, the digitized data are transferred from the disk for storage onto a video cassette. Subsequently, the digitized data from the entire experiment are restored to the Corvus disk from the video cassettes and submitted to statistical analyses with programs resident in the disk. We

conducted analysis of variance (ANOVA) with repeated measures (i.e., trials, blocks, days) on the data of each experiment and post hoc Tukey test for localizing what groups/conditions have contributed to the significant sources of variation (20). The level of significance in all experiments was set at $p < 0.05$.

Experiment 1: Paired CS-US Training

This experiment assessed the effects of nitrous oxide on acquisition of CRs under CS-US pairings. Thirty-two rabbits were randomly assigned to receive one of three doses of N_2O (10 animals received 100% O_2 and 11 each received 33% or 67% nitrous oxide), 30 min prior to and throughout each of six daily experimental sessions. Each session contained 60 CS-US paired trials at a CS-US interval of 400 ms. The intertrial interval was randomly generated with a mean of 60 s (range 50-70 s). On each conditioning trial the offset of a 400-ms tone CS occurred simultaneously with the onset of a 100-ms shock US.

Experiment 2: Unpaired CS, US Training

This experiment aimed at delineating the effects of nitrous oxide on nonassociative processes by presentations of unpaired CS-alone and US-alone stimuli. Twenty-seven rabbits were randomly assigned to one of the three dosages of nitrous oxide (nine animals received 100% O_2, 10 received 33% N_2O, and eight received 67% N_2O) 30 min prior to and throughout each of six daily training sessions. Each session consisted of 120 trials composed of 60 tone-alone and 60 shock-alone trials, so that the total number of stimulus presentations and the duration of the session (60 min) was equal to that employed in the paired CS-US procedure. These trials were presented in a randomized sequence within 20 trial blocks with the restriction that no more than three of the same stimuli were presented consecutively. The intertrial interval was randomly generated with a mean of 30 s (range 20-40 s). The duration and intensity of each stimulus was the same as that described in Experiment 1.

Experiment 3: Effects on US Threshold

This experiment assessed the effects of nitrous oxide on US intensity-UR frequency and amplitude functions. An assessment of anesthetic effect on URs can be obtained from the US-alone trials under unpaired presentations. However, since the US is presented only under a single set of values, it is relatively insensitive to drug effects on the UR. Thirty-one rabbits were assigned to one of three nitrous oxide dosages (10 animals received 100% O_2, 10 received 33% N_2O, and 11 received 67% N_2O) presented 30 min before and throughout each of two daily (56 min) experimental sessions. In each of the sessions, rabbits received 56 US-alone trials at randomized intertrial intervals with a mean of 60 s (range 50-70 s). The 56 trials were composed of eight 100-ms US presentations at each of seven shock-US intensities of 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, and 4.0 mA, each presented once within each of eight, randomized, seven-trial blocks. The frequency, onset latency, peak amplitude, and topography of each UR were recorded.

Experiment 4: Effects on CS Intensity

This experiment was directed at assessing the effects of nitrous oxide on CS intensity relatively independent of learning. Thirty rabbits received 6 days of paired CS-US acquisition training (as in Experiment 1) under 100% oxygen. On the day after the last acquisition session, rabbits were assigned randomly (10 each) to receive one of three nitrous oxide concentrations 30 min prior to and throughout each of two additional sessions in which CS intensity was randomly varied within each of six 10-trial blocks at values of: 45, 50, 55, 60, 65, 70, 75, 80, 85, and 90 dB. The CRs and URs were scored as previously described.

Experiment 5: Effects on Tone-Induced Facilitation of the Nictitating Membrane Reflex

Thirty-two rabbits were assigned to receive one of three nitrous oxide dosages (10 animals received 100% O_2 and 11 each received either 33% or 67% N_2O), presented 30 min before and throughout each of three daily (56 min) experimental sessions. In each session, rabbits received 56 trials at randomized intervals with a mean of 60 s (range 50-70 s). The 56 trials were composed of eight seven-trial blocks. Each block contained, in a randomly determined order, a 100-ms, 2.0-mA US-alone trial and six trials on which animals were exposed to a 400-ms, 84-dB tone-CS at intervals of either 0, 100, 200, 400, 800, or 1600 ms prior to US onset. The amplitude of the NMR for each rabbit on each US-alone trial provided the baseline UR amplitude within the seven-trial block. The amplitude of URs at each CS-US interval for the six tone-shock trials within the seven-trial block were then calculated for each rabbit as a percentage of change from its baseline UR amplitude to determine the extent to which the CS at each CS-US interval served to modify the NM reflex elicited by the US.

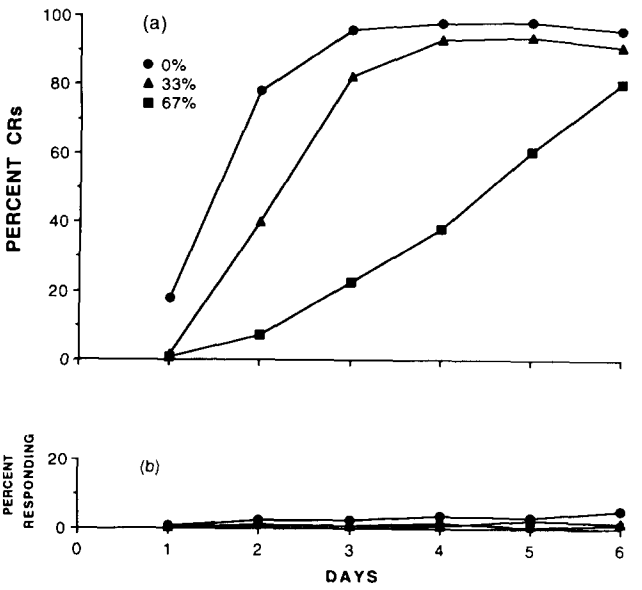

FIG. 1. Effects of nitrous oxide on acquisition of CRs during the paired CS-US training in Experiment 1. (a) The mean percentage of CRs on each of the six acquisition days for animals receiving 100% oxygen (circles, $n = 10$), 33% nitrous oxide (triangles, $n = 11$), and 67% nitrous oxide (squares, $n = 11$). (b) The mean percentage of baseline responses during the 400 ms occurring immediately prior to the tones. Treatment with 100% oxygen is presented as circles, 33% nitrous oxide is presented as triangles, and 67% nitrous oxide is presented as squares. Each point is the mean of nine animals, 10 animals, and eight animals per treatment, respectively.

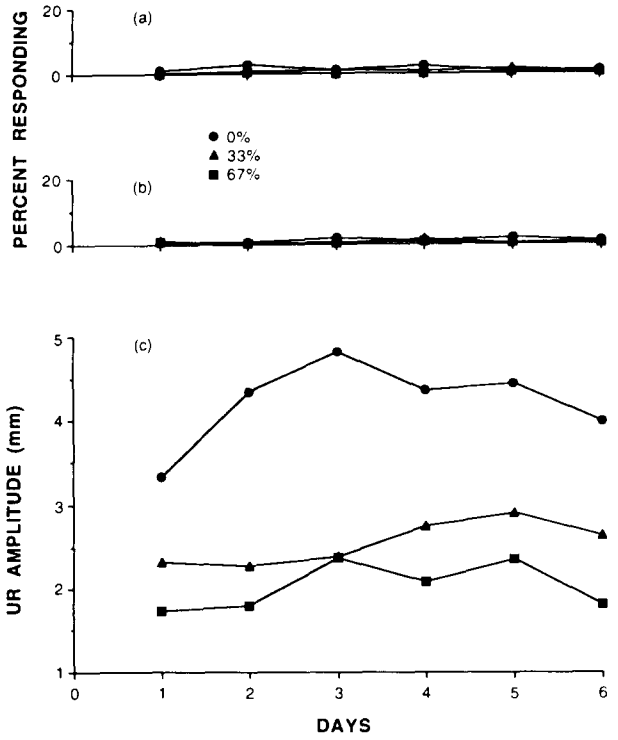

FIG. 2. Effects of nitrous oxide on responding during the 6 days of unpaired CS, US training in Experiment 2. (a) The mean percentage of NMRs occurring during the presentations of the tone. (b) The mean percentage of NMRs during baseline responding, responding in the 400 ms period immediately prior to the tone. (c) The mean amplitude of the unconditioned NMR, in the millimeters of actual membrane extension, elicited by presentations of the 3-mA shock US.

Experiment 6: Does Behavioral Tolerance Develop to Repeated Treatments?

Thirty-one rabbits were randomly divided into three groups. The first group (nine animals) received daily sessions of 100% O_2 for 12 days. The second group (11 animals) received 100% O_2 for the first 6 days and 67% N_2O for the next 6 days. The third group (11 animals) received 67% N_2O for 12 daily sessions. For the first 6 days, all animals received the treatments without any training, and for the next 6 days they received paired CS–US training identical to that described in Experiment 1.

RESULTS

Experiment 1: Paired CS–US Training

Nitrous oxide produced a dose-dependent retardation of CR acquisition to the tone CS as measured by percent CRs and NMR onset latency across the 6 days of conditioning (Fig. 1). An ANOVA on percent CRs revealed significant effects of dose, $F(2, 29) = 27.1$, $p < 0.01$, days, $F(5, 145) = 105.1$, $p < 0.01$, and dose × days interaction, $F(10, 145) = 8.2$, $p < 0.01$. A Tukey follow-up test revealed that percent CRs of both the 0% and 33% nitrous oxide groups were significantly higher than the 67% group ($p < 0.01$). The percent CRs of the 0% group was greater than the 33% group on each day but did not differ significantly. Nitrous oxide also had significant effects upon CR onset latency. The onset latency decreased with daily acquisition sessions; however, nitrous oxide interfered with this decrease. An ANOVA on onset latencies revealed significant effect of dose, $F(2, 29) = 4.4$, $p < 0.05$, days, $F(5, 145) = 14.4$, $p < 0.01$, and dose × days interaction, $F(10, 145) = 4.6$, $p < 0.01$.

Experiment 2: Unpaired CS, US Training

Regardless of the nitrous oxide dosage, the percentage of NMRs across 6 days of unpaired stimulus presentations was low both during (0.9%) (Fig. 2b) and before (0.9%) (Fig. 2c) the tones. An examination of the frequency and latency of NM URs elicited by the 3-mA shock US-alone presentations across the six daily sessions and ANOVA revealed no systematic effects of nitrous oxide dosage. However, the amplitude of URs elicited by the 3-mA shock US-alone presentations revealed significant nitrous oxide dosage effects, $F(2, 24) = 12.28$, $p < 0.01$ (Fig. 2a). A Tukey follow-up test on the amplitude of URs revealed that the URs amplitude of the nitrous oxide groups, both 33% and 67%, were significantly lower than the 100% oxygen control group ($p < 0.01$).

Experiment 3: Effects of Nitrous Oxide on US Threshold

Animals treated with nitrous oxide showed no significant differences from the control group in the percent URs (Fig.

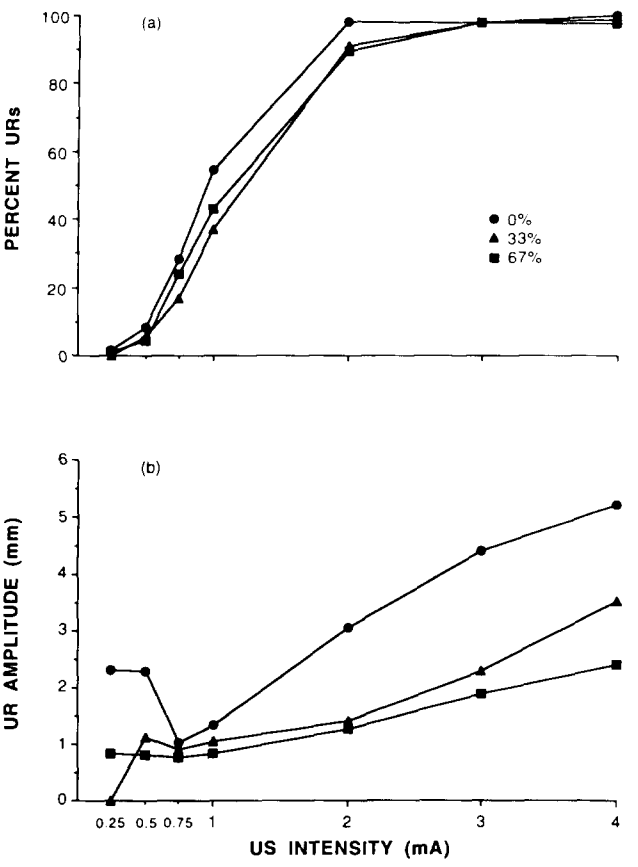

FIG. 3. (a) The mean percentage of URs as a function of the intensity of the shock US. (b) The mean amplitude of URs as a function of the US intensity. Symbols are the same as in Figs 1 and 2. Each point is the mean of 10 animals for the 100% oxygen and 33% nitrous oxide treatments, and 11 animals for the 67% nitrous oxide treatment.

3a) elicited at any US intensity. A US intensity threshold was calculated separately for each animal by interpolating the shock intensity at which URs would have occurred on 50% of the trials. There was no significant difference between the shock intensity thresholds of the control and nitrous oxide groups. However, nitrous oxide had significant effects on the UR amplitude. Figure 3b presents the effects of nitrous oxide dosage on UR amplitude across increasing US intensities. Examination of the figure revealed that the amplitude of URs was an exponential increasing function of US intensity. An ANOVA on response amplitude revealed significant effects of dose, $F(2, 26) = 11.5$, $p < 0.01$, US intensity, $F(6, 156) = 53.7$, $p < 0.01$, and dose × US intensity interaction, $F(12, 156) = 6.1$, $p < 0.01$. A Tukey follow-up test revealed that UR amplitudes of both the nitrous oxide groups were significantly lower than the control group. Thus, nitrous oxide had significant effects on URs by changing the amplitude of the elicited response.

Experiment 4: Effects on CS Intensity

Before determination of CS intensity–CR frequency functions, rabbits had been exposed to six daily acquisition sessions involving the paired presentation of a 84-dB tone CS and a 3-mA shock US during which time only oxygen was inhaled. By the last 2 days of acquisition training, all animals had achieved asymptotic levels of conditioned responding to the CS (96–99%). Figure 4 presents the results obtained when these animals were treated with nitrous oxide and oxygen and tested for CR occurrence to the various intensities of the tone CS. The CR frequency was essentially a monotonic increasing function of CS intensity. The N_2O produced a monotonic decrease in CR frequency across all CS intensities. An ANOVA on the percentage of CRs revealed significant effects of CS intensity, $F(9, 243) = 33.2$, $p < 0.01$, Dose, $F(2, 27) = 21.6$, $p < 0.01$, and CS intensity × dose interaction, $F(18, 243) = 3.1$, $p < 0.01$. A Tukey follow-up test localized the effect of dose to both 33% and 67% doses of nitrous oxide. Moreover, nitrous oxide had significant effects on CR onset latency. The onset latency decreased monotonically with CS intensity, and increased nitrous oxide dosage progressively incremented the onset latency of CRs. An ANOVA revealed

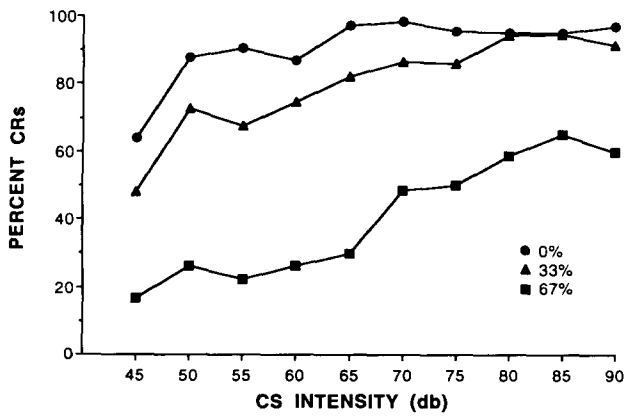

FIG. 4. Effects of nitrous oxide on the CS intensity threshold of a tone for eliciting a CR in Experiment 4. Data are expressed as the mean percentage of CRs as a function of the intensity (dB) of the tone CS. Each point is the mean of 10 animals. Symbols for treatments are the same as in Figs. 1 and 2.

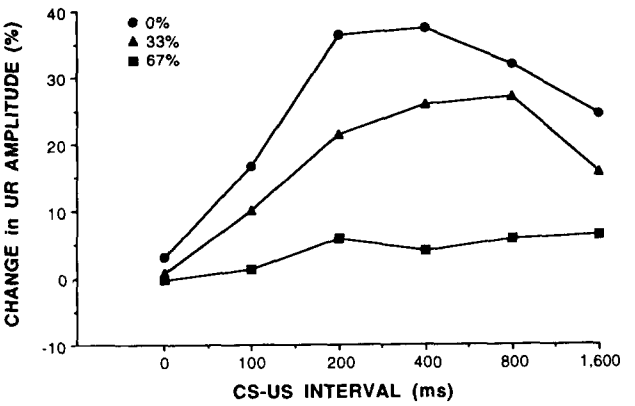

FIG. 5. Effects of nitrous oxide on the facilitation of the nictitating membrane reflex by an auditory stimulus in Experiment 5. The amplitude of the UR elicited by presentation of a 100-ms, 2-mA shock US-alone served as the baseline. The percentage of change in the amplitude of the UR from this baseline value was calculated separately for each animal at each of the tone–shock intervals. Data are presented as the mean percentage of changes in UR amplitude as a function of the time between onset of the tone and shock onset. $N = 10$ for control animals and $N = 11$ for the two nitrous oxide groups. Symbols for treatments are the same as in Fig. 1 and 2.

significant effects of CS intensity, $F(9, 243) = 11.1$, $p < 0.01$, dose, $F(2, 27) = 4.9$, $p < 0.05$, and CS intensity × dose interaction, $F(18, 243) = 2.1$, $p < 0.01$.

Experiment 5: Effects on Tone-Induced Facilitation of the NM Reflex

The amplitude of the UR on trials in which only shock was presented served as the baseline from which percentage changes in the UR produced by the 84-dB tone were calculated. Figure 5 presents the effects of nitrous oxide dosage on reflex modification. The amplitude of URs elicited by the US-alone presentations revealed significant nitrous oxide dosage effects, $F(2, 29) = 4.4$, $p < 0.05$. This result is consistent with Experiment 3. The tone stimulus produced a significant increase in the amplitude of the UR that was a function of the tone–shock interval. In the control group, there was a rapid rise in reflex facilitation that reached asymptotic levels at 400 ms after tone onset. Nitrous oxide significantly reduced the ability of the tone stimulus to increase the amplitude of the UR. An ANOVA revealed significant effects of CS–US interval, $F(5, 145) = 27.5$, $p < 0.01$, dose, $F(2, 29) = 8.3$, $p < 0.01$, and CS–US interval × dose interaction, $F(10, 145) = 5.3$, $p < 0.01$. A Tukey follow-up test revealed that both the nitrous oxide groups demonstrated significantly smaller changes in UR amplitude than the control group ($p < 0.01$). All dosage groups were significantly different at tone–shock intervals from 200 to 400 ms ($p < 0.01$).

Experiment 6: Effects of Repeated Treatments on the Development of Tolerance

The rates of acquisition of CRs, their amplitudes, and their onset latencies were compared among the three treatments (100% oxygen for 12 days, 67% nitrous oxide for 12 days, and 100% oxygen for 6 days and 67% nitrous oxide for 6 days) and across the 6 days of training. An ANOVA on percent CRs revealed significant effects of dose, $F(2, 28) =$

10.06, $p < 0.01$; days, $F(5, 140) = 76.01$, $p < 0.01$, and dose × days interaction, $F(10, 140) = 3.195$, $p < 0.01$. A Tukey follow-up test revealed that all groups were significantly different from each other ($p < 0.05$). The percent CRs of the 100% oxygen control group was greater than the oxygen-nitrous oxide group and the latter was greater than the nitrous oxide-nitrous oxide group (Fig. 6). Analysis of the response amplitude showed no significant differences among the three treatment groups. Finally, ANOVA of the response latencies revealed significant effects of dose, $F(2, 28) = 6.39$, $p < 0.01$; days, $F(5, 140) = 26.24$, $p < .01$, and dose × days interaction, $F(10, 140) = 10.33$, $p < 0.01$. A Tukey follow-up test revealed that the 100% oxygen control group showed significantly lower latencies than the oxygen-nitrous oxide group ($p < 0.05$) and the nitrous oxide-nitrous oxide group ($p < 0.01$). There were no significant differences between the latter two groups.

DISCUSSION

The paucity of animal research on the effects of inhalation anesthetics on basic as well as more complex behavioral processes may be attributable, in part, to the failure of investigators to develop or select appropriate animal models. Classical conditioning of the rabbit NMR has been developed into a model system for the study of drug action because of its ability to:

1. exactly specify the stimulus conditions governing acquisition (and other learning) processes;
2. delineate the associative effects of the variables of interest from their effects on nonassociative, sensory, and motor processes;
3. reveal sensitivity to incremental and decremental effects of drugs on learned behavior;
4. localize drug effects on learned behavior to sensory, associative, and motor processes; and
5. allow the further partitioning of drug action to temporal and motivational processes as well as more complex processes (5,9,21).

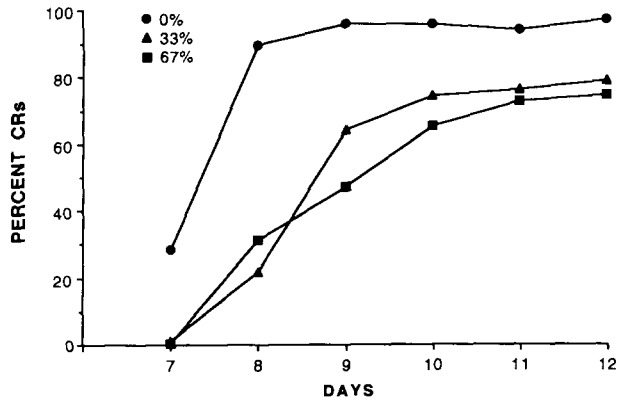

FIG. 6. Effects of nitrous oxide on acquisition of CRs during the paired CS, US training in Experiment 6. Animals were treated with either 100% oxygen or 67% nitrous oxide for the previous 6 days without training. Data are expressed as mean percentage of CRs. Animals treated with 100% oxygen for 12 days are represented by circles ($n = 9$), those treated with 100% oxygen for the first 6 days and 67% nitrous oxide for the next 6 days are represented by triangles ($n = 11$), and those treated with 67% nitrous oxide for 12 days are represented by squares.

The principal findings of the present series of studies using this model were that nitrous oxide:

a) impaired the rate of CR acquisition and overall level of CRs (Experiment 1);
b) had no effect on the NMR base rate, sensitization, and pseudoconditioning, but did impair UR amplitude to a single value of US intensity (Experiment 2);
c) impaired UR amplitude over an extended range of US intensities (Experiment 3);
d) attenuated the intensity of the tone CS and elevated CS intensity threshold (Experiment 4);
e) reduced the ability of the tone stimulus to increase the amplitude of the UR (Experiment 5); and
f) did not induce tolerance with repeated administrations (Experiment 6).

A more detailed analysis was conducted of N_2O's impairment of CR acquisition with a determination of the anesthetic's effect on the initiation of conditioning. The analysis revealed a dose-dependent increment in the number of trials to the occurrence of the first, second, and third consecutive CRs. The dose-dependent effects of N_2O on trials to criterion and the overall level of CRs indicates that the pattern of N_2O's effects was essentially the same before and after CR occcurrence. This similarity suggests that a principal effect of N_2O was to impair the entry of conditioning components (CS, US, and/or UR) into the process governing CR acquisition. Moreover, the absence of any detectable effects of nitrous oxide on NMR base rate or nonassociative (sensitized and psuedoconditioned) NMRs (Experiment 2) further indicates that N_2O operated upon those associative processes governing the acquisition of CRs and their subsequent evocation (performance).

Experiment 2 revealed that nitrous oxide had a significant effect on UR amplitude to a fixed-shock US intensity. Furthermore, in Experiment 3, N_2O affected the psychophysical functions relating US intensity to the amplitude of URs. Hence, nitrous oxide appears to have exercised one of its effects on conditioning through an alteration in the sensory effects of US intensiy and/or motor functioning. At present, we are involved in a series of electrophysiological studies seeking to determine if N_2O's effects are upon the afferent and/or efferent pathways of the UR.

Experiment 4 indicated that, over an extended tone CS intensity range, nitrous oxide decreased tone CS intensity and increased the CS intensity threshold. These effects were obtained postasymptotically, and, therefore, presumably reflected changes in performance (i.e., the probability of evoking CRs). Experiment 5 revelaed that nitrous oxide attenuated reflex facilitation and, by operational definition, decreased the unconditioned excitatory properties of the tone CS. Because the CS's unconditioned excitatory effects would be reduced at the very start of training, N_2O would be expected to impair the CS's entry into conditioning from the beginning of acquisiton training. Hence, the results of Experiments 4 and 5 indicated that N_2O affected the acquisition and performance of CRs in a manner consistent with conditioning theories for which CS intensity affects both the rate of CR acquisition and the postasymptotic level of CR evocation (performance) [e.g., (7,10,14)]. These theoretical expectations are also in agreement with previous rabbit NMR drug studies revealing that scopolamine (9), haloperidol (8), and morphine (15) retarded CR acquisition and the effect was localized to their attenuating tone CS intensity; LSD enhanced CR acquisition while also potentiating tone CS intensity (5).

At a more physiological level of analysis, several studies have suggested that nitrous oxide's hypnotic effects may serve as the mechanism by which it affects learning by altering the intensive properties of the CS. These studies have suggested that nitrous oxide may manifest its hypnotic effects by acting on the reticular formation and blocking EEG arousal thresholds to auditory stimuli (1,2,12). The ability of nitrous oxide to block the tone CS's reflex facilitation has also been postulated to be the basis for its retardant effects on CR acquisition, because heterosynaptic reflex facilitation is presumed to be the basis for the plastic changes that lead to learning (11,18). Therefore, both of the above behavioral and physiological accounts place a crucial role upon those variables affecting conditioning through their effects upon the intensive properties of the CS.

The absence of tolerance to nitrous oxide effects on acquisition parallel the same results in acute experiments in humans (13) and suggest the applicability of the data to experiences with single administrations. Future studies, using classical conditioning procedures, should advance our knowledge on the effects of anesthetics on learning and retention, and should localize their effects upon the neural circuitry. In the words of Thompson and Gluck (19) "In the context of brain stubstrates of learning and memory, classical or Pavlovian conditioning has proved to be the Rosetta stone for both invertebrate and vertebrate preparations."

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