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Does Nitrous Oxide Antagonize Isoflurane-induced Suppression of Learning?

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Background: **A greater MAC fraction of nitrous oxide than isoflurane is required to prevent response to verbal commands and suppress the capacity to learn. Speculating that this difference between these agents may be caused by nitrous oxide's capacity to increase sympathetic activity, we tested the hypothesis that nitrous oxide may antagonize the suppression of learning found with isoflurane.**

Methods: **We administered a combination of isoflurane and nitrous oxide at three subanesthetic test concentrations (0.43, 0.56, and 0.68 MAC) to 24 healthy male volunteers. Assuming additivity of the anesthetics, the first test concentration was selected to suppress learning of new information by 50% (ED_{50} for suppression of learning); the second concentration, to suppress the ability to respond appropriately to verbal command by 50% (MAC-awake); and the third, to provide 1.4 times MAC-awake. Three tests of learning were applied. At each test concentration, we provided 7 answers to "trivial pursuit"-type questions, resulting in a set of 21 answered questions for each volunteer; an additional 7 unanswered questions served as controls. At the highest test concentration, each volunteer also heard two examples from each of two categories (4 words) repeated 30 times (the category-example task), and a message instructing them to touch either their nose or their ear during a specified interval in the postanesthetic interview (the behavior task).**

Results: **The MAC-awake value for the combination of isoflurane and nitrous oxide was 118 ± 4% of the expected value (*i.e.*, the two anesthetics were antagonistic for this effect). Consistent with antagonism, the anesthetic concentration predicted to suppress learning by 50% permitted significantly more learning, and the ED_{50} was 105 ± 2% of that predicted. Neither the category task nor the behavior task demonstrated evidence of learning at 1.4 times MAC-awake.**

Conclusions: **Our results are consistent with an antagonism between nitrous oxide and isoflurane; however, the degree of antagonism is small. (Key words: Anesthesia, depth: awareness; learning; MAC-awake. Anesthesia, inhalational: isoflurane; nitrous oxide. Learning, memory, recall: explicit; implicit. Learning, testing: behavior task; category-example task; Trivial Pursuit-question task.)**

IF inhalational anesthetics share a common mechanism by which they produce anesthesia (unitary theory of narcosis), then, in the absence of a confounding variable, anesthetic effects of various agents should be additive.[1] Additivity may also be expected for other endpoints, such as the suppression of learning (ED_{50} for the suppression of learning) and the ability to respond to command (MAC-awake), if these also share a common mechanism of action.

The level of sympathetic activity may provide a confounding variable. The dose of anesthetic agent required to suppress learning is reported to be influenced by exogenous epinephrine.[2] We hypothesized that increased systemic sympathetic activity during nitrous oxide anesthesia[3,4] may elevate the ED_{50} for the suppression of learning.

Both direct and indirect evidence indicates that nitrous oxide may antagonize its own, and other anesthetics', effects on learning. We previously found that nitrous oxide is less potent (in terms of MAC-equivalents) than isoflurane in suppressing learning.[5] In addition, most studies and case reports documenting unconscious (implicit) and/or conscious (explicit) learning in humans during general anesthesia used anesthetic regimens including nitrous oxide. Types of implicit learning observed include a therapeutic response (decreased requirement for pain medication, earlier discharge, and decreased postoperative morbidity) from intraoperative positive suggestions;[6–8] altered behavioral and verbal responses, as measured by learning tasks (behavior oriented tasks, word completion tasks, and other verbal oriented tests);[9,10] and ev-

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idence of learning demonstrated by hypnosis-assisted recall.[11] Finally, Yli-Hankala *et al.*[12] found electroencephalographic evidence that nitrous oxide opposes the depression of the central nervous system produced by isoflurane.

In the current study, we examine whether nitrous oxide antagonizes the capacity of isoflurane to suppress learning. That is, does concurrent administration of nitrous oxide and isoflurane increase the anesthetic requirement for suppression of intraoperative learning? We also investigate the dose of anesthesia required to inhibit the appropriate response to command (MAC-awake).

Materials and Methods

We prospectively studied 24 healthy male volunteers (aged 22–30 yr) with the volunteers' informed consent, and approval from the Committee on Human Research at the University of California, San Francisco. We administered a combination of isoflurane and nitrous oxide to each volunteer in three sequential subanesthetic concentrations, and tested the capacity to learn and to respond appropriately to verbal command at each concentration. The three anesthetic concentrations were administered in increasing order to replicate the study protocol that defined the values for the ED_{50} for suppression of learning and the ability to respond to command (MAC-awake) for isoflurane alone, and nitrous oxide alone.[5] One learning task that can be used to reveal implicit learning, "trivial pursuit-type questions and answers,"[5,13,14] was administered at all three test concentrations to provide information on the dose-response suppression of learning. Two additional tasks, the category-example task[15] and the behavior task,[9] were administered at the highest test concentration. These three tasks measure learning by eliciting whether intraanesthetic suggestions influence subsequent verbal or physical behavior.

The trivial pursuit-type question-and-answer task[5,13,14] provides the anesthetized subject with answers to some, but not all, of a group of interesting and obscure questions. After anesthesia, the subject is asked to select an answer for each question from a five-option multiple-choice format. The number of correct responses to questions, the answers to which had and had not been supplied during anesthesia, is noted.

The category-example task presents the anesthetized volunteer with four words (two examples each of two categories) repeated 30 times. After anesthesia, the volunteer is asked to give the first three examples that come to mind for each of four categories (two presented during anesthesia and two controls). We noted the number of responses that were the same as the predetermined examples, four of which had, and four of which had not, been presented to the volunteer.

For the behavior task, we instruct the anesthetized volunteer to touch either his ear or his nose many times when we take his blood pressure during the postanesthetic interview, and compare the preanesthetic incidence with the postanesthetic incidence of touching these sites.

Preliminary Tests

A preliminary test was conducted on a control group of approximately 100 students who were similar in age, sex, and education to our study population. To establish an appropriate data base for trivial pursuit-type questions for our study population, we tested 130 questions in a 5-option multiple-choice format. The students were asked to select one answer for each question, to indicate if they believed they knew the correct answer or were guessing, and to rank their interest in each question using a scale of 1 (boring, uninteresting) to 5 (fascinating, "wish I knew the answer"). We selected 28 of the 130 multiple-choice questions for study using the following criteria: (1) the correct answer was selected 10–30% of the time, *i.e.,* with a frequency that approximated chance; (2) each of the five options was selected at least 8% of the time, but not more than 44% of the time; and (3) the correct answer was not indicated as "known" (*vs.* "guessed") by more than 10% of the students. We divided the 28 questions into four groups of 7 questions, with the groups balanced for frequency of correct response and level of interest.

In the preliminary study, we also verified that the examples used by Roorda-Hrdlickova *et al.*[15] for the category-example task (yellow, banana, green, pear) met the same criteria in our study subjects as described in their population. That is, the examples were commonly cited, but not commonly the most popular (*i.e.,* neither first nor second choice) of the categories. Additionally, we selected examples for the categories "vegetables" and "baked goods" that met the above criteria: zucchini, bread, potato, and pie. Examples not necessarily independent of each other (zucchini-bread and potato-pie) were chosen to help evaluate whether "contextual link" accounted for the remarkable results reported by Roorda-Hrdlickova *et al.* (*i.e.,* yellow-ba-

nana and green-pear have a parallel contextual link). In a previous study using examples without such a contextual link, we did not replicate the findings of the studies by these investigators.[16]

Anesthetic Management

All volunteers fasted for the 12 h preceding the study, and a nonparticulate antacid was administered (Alka-Seltzer Gold in 30 ml water) to neutralize gastric contents. A catheter was placed in a vein on the dorsum of the left hand, and lactated Ringer's solution was infused. Monitors included a precordial stethoscope, a pulse oximeter, and an automated blood pressure cuff.

The volunteers breathed 100% oxygen through a clear plastic face mask attached to a semiclosed anesthetic circuit. Nitrous oxide and isoflurane were introduced to maintain an end-tidal concentration of 40% nitrous oxide and 0.06% isoflurane, as measured by mass spectroscopy, and inspired nitrogen was continuously monitored to detect leaks in the delivery system. To prevent contamination of end-tidal samples with inspired gas, dead space was augmented at the sampling port with tubing having an internal volume of 95 ml. An end-tidal concentration of 40% nitrous oxide was chosen, because higher concentrations had produced a high incidence of vomiting in previous volunteer studies.[5] We selected 0.06% isoflurane because, if simple additivity applied, this concentration with 40% nitrous oxide should have produced the ED_{50} for the suppression of learning. This prediction assumed the ED_{50} for suppression of learning by nitrous oxide alone to be 0.50 MAC (52% nitrous oxide),[5] and for suppression of learning by isoflurane alone to be 0.20 MAC (0.256% isoflurane).[5,16] That is, 40/52 + 0.06/0.256 = 100% (table I).

Experimental Protocol

After 15 min at stable end-tidal concentration, the volunteers were told to squeeze the investigator's hand one, two, or three times, and the response was noted. Earphones were then placed on each volunteer, and each was enjoined to listen carefully. One of four tapes containing the answers to 7 of the 28 trivial pursuit-type questions was played. Each answer was given three to five times, with varying syntax, *e.g.*, "Ed Sullivan's wife's name is Sylvia, Sylvia is the name of Ed Sullivan's wife. The wife of Ed Sullivan is named Sylvia." On completion of the taped presentation, the response to command was again tested.

Table 1. Test Concentrations, Predicted Potencies, and Measured Potencies

	Test Concentration		
	First	Second	Third
Anesthetic administered			
Nitrous oxide (%)	40	40	40
+	+	+	
Isoflurane (%)	0.06	0.22	0.38
Potencies predicted (assuming additivity)			
Multiple of the ED_{50} for suppression of learning	1.0	1.63	2.25
Multiple of MAC-awake	0.73	1.05	1.38
Multiple of MAC	0.43	0.56	0.68
Results			
Questions answered correctly (%) (corrected for controls)	67 ± 6	7 ± 5	−5 ± 3
Subjects responding to command (%)	100	77	12.50

The first row provides the subanesthetic concentrations of nitrous oxide and isoflurane that were administered at the three test concentrations. The second row provides the multiples of the ED_{50} for suppression of learning, the MAC-awake, and the MAC that these concentrations were predicted to provide, assuming additivity of the agents. The third row (results) gives the percentage of correct responses at each of the anesthetic concentrations. Note that the first test concentration, predicted to suppress learning by 50% (1.0 times the ED_{50}), allowed a small but significant increase in the amount of learning; the second test concentration, 1.05 times the concentration predicted to suppress response to command by 50%, allowed a greater percentage of correct responses.

ED_{50} = effective dose suppressing 50% of the response.

Next, we administered 0.22% end-tidal isoflurane with 40% end-tidal nitrous oxide for 15 min. Assuming additivity, this yields 163% of the ED_{50} for the suppression of learning, and 105% of the MAC-awake. MAC-awake for nitrous oxide is 0.64 MAC (66.5% nitrous oxide),[5] and for isoflurane is 0.385 MAC (0.486% isoflurane);[5,16] 40/66.5 + 0.22/0.486 equals 105% of the expected MAC-awake, assuming additivity. We tested the response to command, then enjoined each volunteer to listen carefully, placed the earphones, and played a tape with answers to seven additional trivial pursuit-type questions. After playing the tape, we tested the response to command again.

This protocol was repeated using 0.38% end-tidal isoflurane with 40% end-tidal nitrous oxide concentration. Assuming additivity, this combination was equivalent to 225% of the ED_{50} to suppress learning (0.38/0.256 + 40/52), 138% of MAC-awake (0.38/0.486 + 40/66.5), and 68% of MAC (0.38/1.28 + 40/104). In addition to providing seven new answers to questions, we played one of two tapes with examples of categories

(the category-example task), and one of two tapes with a behavioral message (the behavior task).

To avoid testing bias, the order of administration of the four sets of 7 answers was randomized and balanced over the 24 volunteers, allowing each answer set to be used with equal frequency at each of the 3 subanesthetic levels, and as controls.

We discontinued administration of the anesthetic agents and allowed the volunteers to breath 100% oxygen until they regained consciousness. They were interviewed after a 60–90-min recovery period.

The behavioral message was either: (1) "This is Hank Bennett, and this is a special research message for you. When we take your blood pressure in the interview after anesthesia, it is very important that you touch and rub your nose many times. Touching and rubbing your nose is how we will know that you can hear us under anesthesia. It is very important that you touch and rub your nose when we take your blood pressure after anesthesia. Your nose may itch and you will need to touch and rub it, or you might just know to touch and rub your nose many times. In the interview after the anesthesia, touch and rub your nose many times when we take your blood pressure," or (2) The same message with "touch and pull on your ear," substituted for "touch and rub your nose."

For the category-example task, we presented volunteers with two examples for each of two categories (a total of four examples). The examples were spoken at a rate of one every 1.5 s. We used "yellow, banana, green, pear" for the categories "colors and fruit" with one-half of the volunteers. These were the examples used by Roorda-Hrdlickova *et al.*[15] To the other one-half, we presented the examples "zucchini, bread, potato, pie" for the categories "vegetables and baked goods." For each volunteer, the examples not presented served as controls.

To ensure that all messages were clearly audible (normal listening tone) and the appropriate tapes used with each volunteer, an investigator who did not participate in the postanesthetic interview monitored all tape presentations using parallel headphones.

Learning Assessment

"Trivial Pursuit"-Type Questions. Before anesthesia, each volunteer was asked all 28 trivial pursuit-type questions, and was encouraged to answer when they believed they knew the answer or to say "I don't know." (If an answer was known correctly, the question was not included in the assessment of learning.) Volunteers were thus exposed to the questions that would be answered during anesthesia. Volunteers were presented with the answers to 21 of the questions, 7 at each of the three anesthetic test concentrations, with the remaining 7 serving as the controls. After recovery from anesthesia, the 28 questions were presented in a 5-option multiple-choice format. After committing to answers for all 28 questions, volunteers were asked to note whether the answer they selected: (1) was previously known, (2) was heard during anesthesia, or (3) was a guess.

The number of correct responses to questions answered during anesthesia was compared with the number of correct responses to control questions.

Category-Example Task. During the postanesthetic interview, each volunteer was asked to close his eyes and state the first three examples of each of the following categories: colors, fruit, vegetables, and baked goods. The two categories not presented during anesthesia served as controls. Responses were compared with the examples given during anesthesia.

Behavior Task. During the preanesthetic and postanesthetic interview, the interviewer measured each volunteer's blood pressure using an automated blood pressure cuff. We discreetly noted the number of times that each volunteer touched his ear and nose during the interval beginning with the statement, "I am going to take your blood pressure now," and ending with the removal of the blood pressure cuff. During anesthesia, volunteers were presented with instructions to touch one of these sites during postanesthetic blood pressure measurement. The frequency with which each site was touched during the pre- and postanesthetic interviews was compared.

As a further measure of implicit learning, we read both behavioral messages and each set of category examples to volunteers, asking them to guess which sounded most familiar. We noted the incidence with which they correctly identified the information presented during anesthesia as "most familiar." This task and the previous tasks may combine elements of explicit and implicit learning, depending on the degree of recognition and the definition of recognition as a measure of explicit or implicit learning.

Data Analysis

Statistical analyses were performed as follows.

Trivial Pursuit-Type Questions. We subtracted 20% from each volunteer's score, the percentage of questions answered correctly, to account for random

selection of the correct answer (in the five-option multiple choice questions). The result was then divided by 0.8, thereby producing a scaling in which 20% equated to 0%, and 100% remained 100%. We applied a two-tailed, one-sample t test to the first anesthetic test concentration (equal to 100% of the ED_{50} for suppression of learning assuming additivity) to determine if the amount of learning at this concentration was different from that predicted (*i.e.*, different from 50%). In addition, at each anesthetic level, the percentage of questions answered correctly by each volunteer was compared with their control percentage using the Wilcoxon matched-pairs signed-ranks test, with statistical significance accepted at $P < 0.0167$ (Bonferroni correction for multiple comparisons). Finally, we fit a sigmoid equation using BMDPAR-Derivative-Free Nonlinear Regression§ to calculate the ED_{50} for suppression of learning (*i.e.*, the concentration of isoflurane that permitted [or suppressed] conscious recall of 50% of the examples presented).

Category-Example Task. The number of correct answers ("hits") for the presented examples was compared with the number of "hits" for the control examples using the Wilcoxon matched-pairs signed-ranks test, with statistical significance accepted at $P < 0.05$.

Behavior Task. For each testing interval (during blood pressure measurement), the number of times the volunteer performed the control behavior was subtracted from the number of times he performed the experimental behavior. This difference (between the incidence of experimental and control behaviors) during the preanesthetic interview was compared with the difference during the postanesthetic interview using the Wilcoxon matched-pairs signed-ranks test, with statistical significance accepted at $P < 0.05$.

Finally, the ability of the volunteers to guess which behavior message and which set of category examples were presented was analyzed by chi-square analysis, with statistical significance accepted at $P < 0.05$.

Response to Command: MAC-Awake. We determined MAC-awake using the method reported in the study that defined the values for each individual anesthetic.[5] That is, in most cases, an individual MAC-awake value was calculated for each volunteer as the average of the anesthetic concentrations bracketing the loss of the ability to respond to command. In three volunteers, MAC-awake was taken as the anesthetic test concentration at which both appropriate response and absence of appropriate response were obtained at different times. Two volunteers responded appropriately at the highest anesthetic test concentration. Calculation of the MAC-awake for these two individuals required the assumption that a further equal step increase in anesthetic concentration (*i.e.*, an increase of isoflurane to 0.54% in the presence of 40% nitrous oxide—equivalent to 81% of MAC) would have prevented response. The mean MAC-awake value for the group was compared with the value predicted assuming strict additivity of anesthetics using a two-tailed, one-sample t test, with statistical significance accepted at $P < 0.05$.

In addition, we fit a sigmoid equation using BMDPAR-Derivative-Free Nonlinear Regression (see above) to confirm the value for MAC-awake with an analysis that better accounted for the two volunteers who continued to respond at the highest anesthetic test concentration.

Results

The MAC-awake for the combination of isoflurane and nitrous oxide was 118 ± 4% (mean ± SEM, 95% confidence limits 113–122%) greater than the value predicted by simple additivity, $P < 0.001$, indicating that the two anesthetics are antagonistic for this effect (fig. 1).

Learning, as measured by the trivial pursuit-type question task, was suppressed in a dose-related fashion.

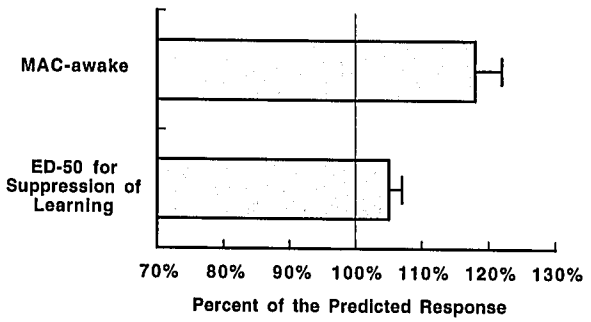

Fig. 1. One hundred percent on the x-axis represents the anesthetic concentration predicted (assuming additivity) to provide a 50% suppression (ED_{50}) for response to command (MAC-awake) and for learning. MAC-awake was 118 ± 4% (mean ± SEM), and the ED_{50} for suppression of learning was 105 ± 2% (mean ± SEM) of the value predicted by additivity. That is, the combination of nitrous oxide and isoflurane allowed more correct responses than would have been predicted, based on the potency of each anesthetic alone, to suppress these end-points.

§ Ralston M: Derivative-Free Nonlinear Regression: BMDP Statistical Software Manual. Edited by Dixon WJ. Berkeley, University of California, 1990, pp 396–422.

Control questions were answered correctly with a frequency equal to random guessing (19 ± 3%, mean ± SEM). After normalizing the data to account for baseline guessing (see data analysis), we found that 67 ± 6% (mean ± SEM) of the questions were answered correctly when the answers were provided at 0.43 MAC. That is, the amount of learning at 0.43 MAC was statistically greater than predicted (*i.e.*, greater than 50%), $P = 0.01$. Learning at the other anesthetic test concentrations was not significantly different from zero: 7 ± 5% (mean ± SEM) when the answers were provided at 0.56 MAC, and −5 ± 3% (mean ± SEM) when the answers were provided at 0.68 MAC (fig. 2). The ED_{50} for the combination of nitrous oxide and isoflurane was 0.45 MAC (95% confidence limits 0.44–0.47 MAC), a significant, but extremely small, increase from the predicted ED_{50} of 0.43 MAC (fig. 1).

At the second subanesthetic concentration (40% nitrous oxide + 0.22% isoflurane, for a combined equivalent of 0.56 MAC), although 77% of volunteers appropriately followed commands, the amount of learning was statistically indistinguishable from control, $P = 0.2$ (Wilcoxon signed-rank test). However, 7 of the 24 volunteers remembered (*i.e.*, showed cued recognition of) at least one answer presented at this concentration (0.56 MAC), 2 of these 7 recalling at least one answer spontaneously. One of the seven volunteers recalled hearing two of the seven answers presented, and correctly guessed the answers to three more questions (total five of seven questions correct). This volunteer's MAC-awake value was 155% of expected, and he followed commands appropriately at 40% nitrous oxide + 0.38% isoflurane (combined equivalent to 0.68 MAC). All seven volunteers demonstrating some learning at 0.56 MAC also followed commands appropriately at this test concentration. No volunteer demonstrated learning of answers to questions at 0.68 MAC.

Category-Example Task

When asked to list three examples for each category—color, fruit, vegetables, and baked goods—eight volunteers listed the presented examples more frequently than the control examples, ten listed the control examples more frequently, and six listed presented and control examples equally ($P = 0.93$, Wilcoxon signed-rank test). The volunteers who heard the examples "yellow, banana, green, pear" did not demonstrate learning better than those who heard the alternate set of examples. When asked to guess which set of examples sounded most familiar, 14 of 24 volunteers guessed the presented examples ($P > 0.5$ by chi-square analysis).

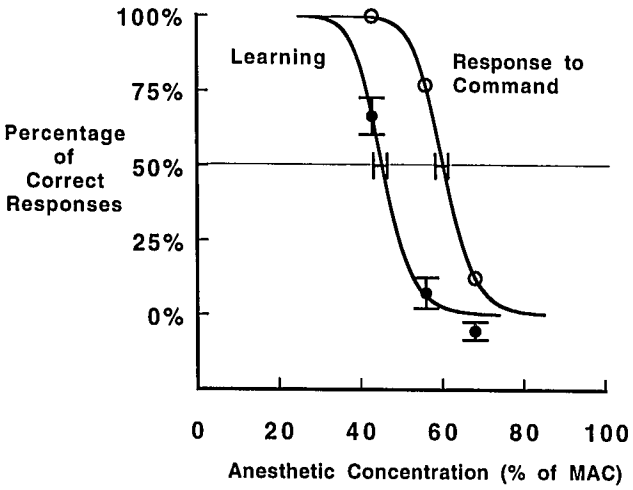

Fig. 2. At each anesthetic test concentration, the percent of questions answered correctly (closed circles), and the ability to follow commands (open circles), are graphed as a function of increasing anesthetic concentrations. Nonlinear regression analysis (see text) was used to fit a sigmoid curve to the data. There is a steep dose response for suppression of both the capacity to learn and the response to command. The anesthetic concentrations required to suppress response to command are greater than the concentrations required to suppress the capacity to learn. Error bars in the y-direction represent the SEM for learning. Error bars in the x-direction represent the 95% confidence limits around the ED_{50}s for learning and for response to command.

Behavior Task

The pre- and postanesthetic incidence (corrected for control) of touching the suggested feature (nose or ear) were not significantly influenced by the behavior message ($P = 0.80$, Wilcoxon signed-rank test). When asked to guess which behavioral message sounded most familiar, 12 of 24 volunteers guessed the presented message ($P > 0.5$ by chi-square analysis).

Side Effects

Inhalation of isoflurane and nitrous oxide at the test concentrations resulted in the need to abort anesthetic administration in three volunteers: two because of persistent retching, and one because of profound anxiety. Consequently, 27 volunteers were required to complete the anesthetic protocol with 24. Pilot studies in a similar population using greater than 40% nitrous oxide with isoflurane were difficult to complete because of the high incidence of nausea and vomiting.

Maintaining the nitrous oxide concentration at 40% only slightly reduced the high incidence of nausea and vomiting. Nine of 27 volunteers vomited during anesthetic administration, and remained nauseated in the immediate recovery period. When retching interfered with anesthetic delivery, an additional 5–10 min of equilibration time was added to ensure stability of anesthetic concentration. One volunteer experienced a marked excitation phase at the highest anesthetic concentration, which we resolved by increasing the anesthetic concentration, then returning to the target concentration for a full 15-min equilibration period after attainment of a stable end-tidal concentration. At the lowest test concentration, two volunteers complained of slight anxiety, which resolved spontaneously.

Discussion

In MAC equivalents, nitrous oxide is less potent than isoflurane in preventing response to verbal commands and in suppressing the capacity to learn.[5] This difference may result from nitrous oxide's capacity to stimulate sympathetic activity, or from a unique quality of the agent. Yli-Hankala *et al.*[12] found electroencephalographic evidence that nitrous oxide opposes the depression of the central nervous system produced by isoflurane. Consistent with these reports, we found that nitrous oxide also antagonizes the capacity of isoflurane to suppress response and learning. MAC-awake for isoflurane with 40% nitrous oxide was greater than would have been expected from additivity. The difference, however, was small. Some portion of this small effect may be attributable to our underestimating the MAC-awake value for nitrous oxide. The study defining MAC-awake for nitrous oxide did so by extrapolation of their data.[5] If the true MAC-awake for nitrous oxide is greater than 0.64 MAC, this would contribute to our finding less than the expected effect from the anesthetic concentrations we delivered.

Consistent with antagonism, combined isoflurane and nitrous oxide at 0.43 MAC permitted more learning, as defined by the trivial pursuit-type question test, than was predicted by simple additivity of the anesthetics. However, the ED_{50} of the combination was only slightly greater than predicted. The slope of the dose-response suppression of learning by inhalational anesthetics is steep; thus, one would expect large variations in the amount of learning with small changes in anesthetic concentrations.

A second potential limitation is the precision with which a bracketing technique can define MAC-awake. Precision is determined by two factors: (1) the incremental increase in concentration (step-size) and (2) the number of steps. Because MAC-awake equals the concentration midway between the values permitting and preventing response, the maximum error must be less than one-half the step size. In the current study, this equaled 0.065 MAC or 0.165 MAC-awake for the combined values for isoflurane and nitrous oxide. The error would be considerably less than this if the values for MAC-awake were reasonably distributed across steps (as in the current study). In addition, in a separate analysis, we used nonlinear regression to fit a sigmoid curve to the data, and confirmed our value for MAC-awake.

The steep dose-response curve makes any variability in the values on which we based our predictions more likely to cause a type I error in our findings. It should be emphasized that the values on which we based our predictions for both MAC-awake and the ED_{50} for suppression of learning have a variability that is not accounted for by a one-sample *t* test. In addition, the values on which we based our predictions were previously defined. Conditions in which our study and the previous studies were performed may not be truly identical, despite our controlling for the same anesthesia research group, using the same equipment, the same volunteer profile, and a similar protocol. Therefore, small differences between predicted values and actual values that are statistically significant may not represent a real difference.

The category-example task, applied only at the deepest level of anesthesia, failed to demonstrate learning. This contrasts with results reported by some investigators,[15,17] but confirms results that we and others previously found.[10,16] We had hoped that two modifications to our earlier study design[16] would improve the sensitivity of this task. First, we used the exact examples applied by Roorda-Hrdlickova *et al.* (yellow, banana, green, pear), after ensuring that these examples met our selection criteria. Second, we drew new category examples (zucchini, bread, potato, pie) from our preliminary tests in volunteers. Both example sets demonstrated the same contextual link (zucchini-bread, like yellow-banana, are examples not wholly independent of each other), allowing us to evaluate whether such a link may have increased the sensitivity of the test. The category-example test yielded no evidence of learning during anesthesia in our study population.

The behavior task also failed to demonstrate implicit learning. Similar to the category-example task, this finding contrasts with results reported by some investigators,[9,13,18] but confirms results we and others previously found.[5,16] We were concerned that our earlier attempts to show learning with the behavior task may have been obscured by our loosely defining the pre- and postanesthetic intervals during which performance of the behavior was scored. In the current study, we set well defined limits on the time during which performance of the behaviors would be monitored. The delineated period, the interval for measuring a single blood pressure, was consistent in length, and started and finished with well defined cues to the subject.

Despite no evidence of learning, the behavior task yielded interesting anecdotes. One volunteer, who had been given the message to "touch your nose," appeared to respond accordingly. He had been sitting with his hands folded across his lap during the postanesthetic interview, but abruptly altered his posture when we began to take his blood pressure. He stared directly into the eyes of the (blinded) interviewer, smiled, and vigorously rubbed his nose. The interview progressed without further facial touching. After the official portion of the interview was complete, the interviewer who was so impressed by the volunteer's behavior again took a blood pressure measurement. Again the volunteer touched his nose. When asked to guess which instructions seemed most familiar, the volunteer identified "touch your nose" as familiar. Results with another volunteer were equally remarkable. The second volunteer touched and rubbed his nose each time during three (one extra) postanesthetic blood pressure measurements. He had not touched his nose during the preanesthetic interview, nor his ear (the alternative behavior) during the pre- or postanesthetic interviews. However, he had received the message to touch his ear. Both of these volunteers had low MAC-awake values, failing to respond appropriately to verbal command at the second anesthetic test concentration. Neither appeared to have any explicit knowledge of the instructions.

Our inability to demonstrate an influence on postanesthetic behavior contrasts with the results reported by Bennett for patients.[9] Perhaps these differences result from differences in study populations. Another possibility is that, in Bennett's study, end-tidal anesthetic concentrations were not monitored, and a level of anesthetic that permitted learning may have been reached.

Assuming that individuals with higher MAC-awake values may have demonstrated more learning (as measured by the trivial pursuit task) than individuals with lower MAC-awake values, we applied a linear regression analysis to determine if the amount of learning at each anesthetic test concentration was related to the MAC-awake value for each volunteer. We found no such correlation. Learning was not increased in volunteers with higher MAC-awake values. Overall, suppression of learning occurred at anesthetic concentrations less than MAC-awake. This agrees with our previous findings for inhaled anesthetics, that suppression of learning is achieved at or below anesthetic concentrations sufficient to prevent appropriate response to command.[5,16]

Our results in volunteers should be extrapolated with caution to patients undergoing surgery. The attendant apprehension, and the infliction of noxious stimulation, inherent in a surgical procedure may (or may not) alter the capacity to learn.

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