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Effects of Isoflurane and Nitrous Oxide in Subanesthetic Concentrations on Memory and Responsiveness in Volunteers

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Awareness, defined as conscious memory during anesthesia, has been a problem in anesthesia practice. To determine the effect of isoflurane and nitrous oxide (N_2O) on memory, 17 healthy adult volunteers were randomly assigned to receive isoflurane or N_2O and received the alternate agent 1–2 weeks later. Each volunteer was studied at four end-tidal concentrations of each agent, consecutively 0.15, 0.3, 0.45, and 0.15 times the minimum alveolar concentration (MAC) for isoflurane or 0.3, 0.45, 0.6, and 0.3 times MAC for N_2O. After 15-min equilibration at each end-tidal concentration, volunteers were tested for voluntary response to command and were presented with verbal information to be recalled after anesthesia. Volunteers were interviewed on the day after the study and tested for conscious and unconscious memory of the information presented during anesthetic administration. MAC-awake (the end-tidal concentration preventing voluntary response in 50% of volunteers) was 0.38 (0.35–0.42) times MAC for isoflurane and 0.64 (0.61–0.68) MAC for N_2O (means, 95% confidence limits), indicating isoflurane to be more potent than N_2O in suppressing voluntary response ($P = .0001$). Memory data were analyzed in 12 volunteers who completed the study and in whom the allocation of information to be recalled was counterbalanced among agents and concentrations of agents. Memory was decreased by increasing concentrations of both agents. Conscious memory of the information presented during anesthetic administration was prevented by 0.45 MAC isoflurane but not completely prevented by 0.6 MAC N_2O. Unconscious memory (defined as memory of information without conscious recognition) occurred during administration of both agents and was prevented by 0.45 MAC isoflurane but not by 0.6 MAC N_2O. Isoflurane was more potent in suppressing memory than MAC-equivalent concentrations of N_2O. Using models of the relationship between dose of agent and suppression of memory, a dose of both agents was estimated that suppressed memory by 50% (ED_{50}). The ED_{50} was 0.20 MAC for isoflurane (95% confidence intervals, 0.15–0.25), and 0.50 MAC for N_2O (95% confidence intervals 0.43–0.55). We conclude that isoflurane and N_2O suppress memory in a dose-dependent manner, and that isoflurane is more potent in preventing memory and voluntary response to command than MAC-equivalent concentrations of N_2O.

(Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: isoflurane. Memory: learning, awareness.)

AWARENESS, DEFINED AS conscious memory of events during anesthesia, has been a recurrent problem in anesthetic practice since the introduction of neuromuscular relaxants.[1–3] Although low concentrations of volatile agents decrease or prevent conscious memory,[4–6] the exact anesthetic concentrations required are not known.

Memory may be considered to be conscious (explicit) or unconscious (implicit). Conscious memory includes spontaneous recall (occurring without the aid of a specific cue) and recognition memory (occurring with the aid of a cue). Unconscious memory is manifest by altered performance or behavior due to unremembered experiences.[7,8] By definition, general anesthesia abolishes conscious memory, but whether it also abolishes unconscious memory of verbal information is controversial. Several studies indicate that such unconscious memory may occur during general anesthesia, as shown by the effect of intraoperative suggestion on postoperative behavior[9–11] or on tests of implicit memory.[10,12,13] Other methodologically similar studies, however, demonstrate no unconscious memory.[14–17]

Unconscious memory of events during anesthesia may be important because negative information (*e.g.*, pessimistic statements about a patient's prognosis or disparaging comments about the patient), could have deleterious psychologic effects postoperatively.[18,19] Conversely, positive information (*e.g.*, encouraging a rapid recovery and feelings of well-being postoperatively), presented at a time when patients may be uniquely suggestible,[20] may decrease postoperative analgesic requirement and shorten hospital stay.[11,21]

Most studies documenting unconscious memory report only a weak effect, with memory demonstrated in some, but not all, subjects. One reason for these findings may be that different anesthetic agents or different doses of anesthetic agent were used within studies. Failure to control these variables makes it difficult to determine whether the use of specific agents or even inadequate anesthesia may have contributed to memory of events during anesthesia.

Most studies used nitrous oxide (N_2O) as a component of anesthesia. As sole anesthetic agent (with oxygen), N_2O does not reliably prevent conscious memory.[4,5,22] Tolerance may occur to the analgesic effects of N_2O,[23–25] although there is conflicting evidence as to whether toler-

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ance may occur to its amnesic and anesthetic effects.[23,26] Additionally, N_2O is capable of stimulating as well as depressing the central nervous system[27–29] and might thereby antagonize the depressant effects of volatile anesthetics. Possibly, N_2O differs from the potent volatile agents in its ability to prevent memory.

Similarly, the effect of anesthetic concentration in preventing unconscious memory is unclear. Some studies have been unable to show an effect of anesthetic dose or "depth" in preventing unconscious memory.[10,30]

We have sought to resolve some of these questions by investigating whether isoflurane and N_2O in concentrations that are equivalent in terms of MAC (minimum alveolar concentration of agent required to prevent movement in response to surgical incision in 50% of the population) are equipotent in preventing memory, whether these anesthetic agents decrease conscious and unconscious memory in a dose-related fashion, whether unconscious memory may occur at concentrations of anesthetic agent that prevent conscious memory, and whether tolerance develops to either agent.

Methods

We studied 17 healthy male volunteers (age, 18–34 yr; mean, 25 yr) with the approval of the institutional Committee on Human Research and with informed consent. Most volunteers were medical or dental students, but none were familiar with anesthetic practice. Subjects were interviewed on the day of the study, asked 20 obscure but interesting general knowledge questions (*e.g.*, "What is the blood pressure of an octopus?"), and told they would hear the answers to these questions and a special research message during administration of the anesthetic agents. An observer had the sole task of counting the number of times the subject touched his nose and ear with his hand during the interview, without the knowledge of the subject.

Subjects were fasted, and an antacid was administered before the study to neutralize gastric contents. The electrocardiograph, arterial blood pressure (noninvasive), and arterial hemoglobin oxygen saturation were monitored continuously. With a nose-clip preventing nasal inspiration of room air, subjects breathed 100% oxygen from a semiclosed anesthetic circuit via a mouth piece. Gas was drawn continuously from a sampling port at the mouth piece for analysis. A dead space of 100 ml separated the sampling point from the anesthetic circuit to prevent contamination of end-tidal samples with inspired gas. End-tidal concentrations of isoflurane, N_2O, and carbon dioxide were measured by a mass spectrometer with a 90% response time of less than 0.15 s and that was automatically calibrated by standard gas mixtures at 1-hr intervals.

After the subject breathed oxygen for 3 min, either isoflurane or N_2O (randomized and counterbalanced among subjects) was introduced. Subjects were not blinded as to the agent being administered because of the difficulty in concealing the odor of isoflurane. The inspired concentration was adjusted to achieve specific end-tidal concentrations of each agent. For isoflurane, the end-tidal concentrations achieved were consecutively 0.15, 0.3, 0.45, and 0.15 times the minimum alveolar concentration (MAC), and for N_2O, 0.3, 0.45, 0.6, and 0.3 times MAC. We chose these concentrations of N_2O and isoflurane because previous data suggested they ranged over the concentrations that prevented voluntary response and explicit memory.[31,32] MAC for isoflurane was assumed to be 1.28% in this age group,[33] and MAC for N_2O 1.04 Atm.[34] After maintaining each end-tidal concentration for 15 min to allow equilibration between end-tidal, arterial, and cerebral partial pressures of anesthetic agent, subjects were tested for their ability to respond purposefully to a verbal command by asking them to open their eyes and to squeeze an observer's fingers a stated number of times. At each end-tidal concentration they received a taped message (via headphones) containing the answers to a set of five of the general knowledge questions (randomly allocated) that had been presented before the study began. Each answer was repeated three times. At 0.45 MAC of both agents, subjects also received a message instructing them to touch either their ear or their nose (randomized and counterbalanced between agents) during their postoperative interview. Following recovery from the effects of the anesthetic agents, subjects were allowed to leave.

On the day after the study, subjects were interviewed by an investigator unaware of which answers had been presented during anesthesia. A standardized interview was used to assess memory of verbal material in the following categories: 1) spontaneous recall (uncued conscious memory); 2) total memory, including conscious and unconscious memory; and 3) recognition memory (cued conscious memory).

Subjects were asked, "What do you remember hearing during the experiment yesterday." Spontaneous recall was judged to have occurred when both the question and correct answer were recalled. They were presented with the same 20 general knowledge questions with five multiple-choice answers for each question (one correct and four incorrect "distractor" answers). They were asked to choose or guess the correct answers to all questions. After choosing an answer to all questions, subjects were asked to state whether answers were chosen either from previous knowledge or because they recognized the answer from the study or as a guess. Unconscious memory was assumed to exist if guesses from a particular concentration were correct significantly more often than answers to control questions (see below).

Ear and nose touches during the follow-up interview were counted by a dedicated observer who was unaware of which behavioral message the subject had received. Subjects were not informed that these behaviors were being noted.

Between 1 and 3 weeks after the first study, the experiment was repeated with the alternate agent (N_2O or isoflurane) using the same protocol as in the first study and 20 new general knowledge questions. The alternate behavioral message (to touch the nose or ear) was presented at 0.45 MAC.

At one concentration of one agent, no answers were provided so that one set of five questions was unanswered and seven sets were answered. The number of correct answers to this set of control questions provided a measure of the baseline rate of answering questions correctly (from general knowledge and guesses) and was used as a control for comparison with each of the seven sets of questions answered during anesthetic administration. Sets of questions were randomized and counterbalanced among agents and concentrations of agents. The set of unanswered (control) questions was similarly rotated among all sets of questions, all anesthetic concentrations, and both agents in a randomized and counterbalanced manner. Sets of questions were allocated to a particular anesthetic concentration or used as the control questions a maximum of two times. At each anesthetic concentration, a maximum of two subjects were not presented with answers.

Cardiovascular and respiratory data were compared between the two agents at MAC-equivalent (0.3 and 0.45) doses by paired *t* test. The incidence of vomiting while breathing the anesthetic was compared between agents by Fisher's exact test. The relationship between dose of anesthetic agent and the incidence of vomiting was assessed by regression analysis. MAC-awake (the dose of anesthetic preventing voluntary response in 50% of the population) was assumed to be midway between concentrations that permitted and prevented voluntary response in each individual. MAC-awake was compared between agents by paired *t* test.

The time taken to recover fully from anesthesia was defined subjectively by the subjects, either by volunteering the information during the interview or in response to direct questioning. The time to full recovery was compared between isoflurane and N_2O using Wilcoxon's matched pair test.

Questions answered correctly in the interview before the experiment (5% of the total) were excluded from subsequent analyses to increase the sensitivity of our memory task. Memory was judged to exist when the number of correct answers from a particular anesthetic concentration was significantly greater than correct answers to the five control (unanswered) questions (Wilcoxon's matched pair test). The effects of isoflurane and N_2O on memory were compared by comparing numbers of answers correct after presentation at MAC-equivalent (0.3 and 0.45) concentrations of the agents (Wilcoxon's matched pair test). Correct answers were compared between answers presented during the early and late administrations of the lowest concentration of each agent to test for the effects of prolonged anesthetic administration (Wilcoxon's matched pair test).

The potency of the two agents in preventing memory was compared using probit analysis of the dose-response relationship at three doses. (See Appendix for a detailed description of this statistical analysis). Data from the later dose of the lowest concentrations of the two agents (0.15 MAC isoflurane and 0.3 MAC N_2O) were excluded from this analysis because of the possible confounding effect from vomiting that occurred at the preceding higher dose of N_2O. Values and 95% confidence limits were estimated for 1) the difference between doses of the two agents (as MAC-equivalents) that had the same effect in suppressing memory and 2) the doses of each anesthetic at which the percentage of questions answered correctly were 5% and 50% above baseline. Conceptually, these are the doses of anesthetic preventing memory of almost all (ED_{95}) of and 50% (ED_{50}) of the answers presented.

Conscious recognition was defined by the percentage of answers recognized as having been presented during the study. Correct answers that were not consciously recognized in the follow-up interview were assumed to have been answered from unconscious memory or by chance. Unconscious memory was assumed if questions not consciously recognized were nevertheless answered correctly significantly more often than control questions (Wilcoxon's matched pair test).

The relationships between concentration of anesthetic agent and memory, spontaneous recall, and conscious recognition were assessed by regression analysis (Spearman's rank order correlation coefficient).

The effect of the behavioral message was assessed by comparing numbers of touches of directed and nondirected parts by each subject during the follow-up interview (Wilcoxon's matched pair test). In all analyses, a *P* value of <0.05 was considered statistically significant.

Results

Three subjects withdrew from the study because of severe vomiting (n = 2) or dysphoria (n = 1) while receiving N_2O. Two other subjects were played tapes of answers during anesthesia that did not follow the randomization schedule. We excluded memory data from these five subjects from analysis, leaving memory data from 12 subjects for analysis. We used data for nausea, responsiveness, and ear or nose touching from all 17 subjects.

The time to establishment of a stable end-tidal concentration when changing concentrations was 3–5 min for N_2O and 5–10 min for isoflurane. Equilibration continued for 15 min after achieving a stable end-tidal concentration. Assessment of response to verbal command and presentation of answers took 4–5 min at each concentration. Thus, the time between presentation of each set of answers was approximately 25 min.

Isoflurane significantly decreased mean blood pressure from baseline levels, but N_2O did not. Neither agent altered heart rate. Isoflurane had a dose-related depressant effect on ventilation, as measured by an increase in end-tidal carbon dioxide tension. N_2O had a dose-related emetic effect with a statistically significant correlation between concentration of N_2O and the occurrence of vomiting (fig. 1). Although the incidence of vomiting at equal MAC (0.3 and 0.45) concentrations did not differ between the two agents, the incidence of vomiting at levels that comparably suppressed responsiveness to command (*vide infra*) was 6% for isoflurane and 70% for N_2O ($P = .001$). Twenty-one percent of subjects vomited during recovery from N_2O, but none after isoflurane. The time taken to recover fully was 2 hr after isoflurane and 7 hr after N_2O (medians, $P < 0.05$).

Isoflurane was more potent than MAC-equivalent concentrations of N_2O in preventing voluntary response to verbal command (fig. 2). MAC-awake could not be calculated precisely because some volunteers responded at the highest concentrations administered. However, if we assume that those subjects who responded voluntarily to command at the highest concentration would not have responded at the next step (0.6 MAC isoflurane and 0.75 MAC N_2O), then MAC-awake is 0.38 (0.35–0.42) for isoflurane and 0.64 (0.61–0.68) for N_2O (means, 95% confidence limits, $P = .0001$). The value for N_2O may underestimate the true value.

The baseline rate of answering correctly (the percentage of control questions answered correctly out of five

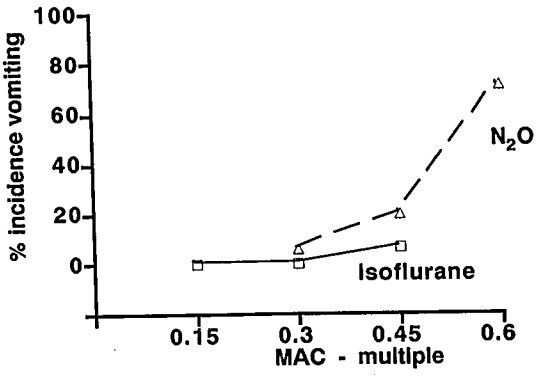

FIG. 1. Percentage of subjects vomiting at each concentration of N_2O and isoflurane.

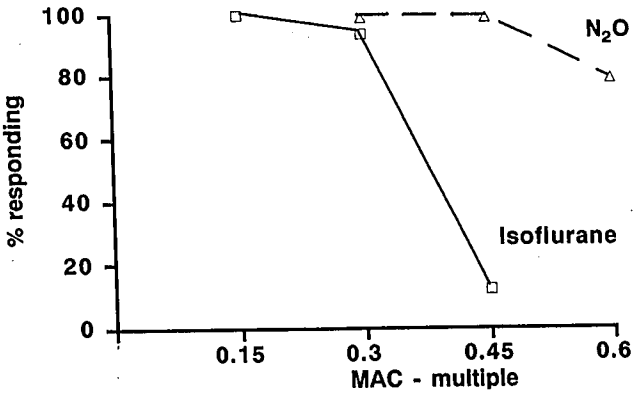

FIG. 2. Percentage of volunteers responding purposefully to verbal command at each concentration of isoflurane and N_2O.

possible choices in the follow-up interview) was 20% (median; quartiles, 0–20%). Memory of answers presented at 0.3 MAC N_2O was almost 100% (fig. 3). Isoflurane and N_2O decreased memory in a dose-related fashion from this high level, and there was a statistically significant negative correlation between concentration of both agents and prevention of memory. Memory of presented answers was significantly greater than control answers for all doses of both agents except 0.45 MAC isoflurane (fig. 3). Although the median values of correct answers were the same for 0.3 MAC isoflurane and control answers (20%), correct answering was significantly greater at 0.3 MAC isoflurane because of the greater interquartile range at this concentration (0–64% *vs.* 0–20%) (fig. 3).

Isoflurane was more potent than MAC-equivalent concentrations of N_2O in preventing memory. Fewer answers presented at 0.3 and 0.45 MAC isoflurane were answered correctly in the follow-up interview than answers from the same MAC-multiples of N_2O ($P < 0.05$) (fig. 3). MAC-multiples of N_2O and isoflurane that were equipotent in

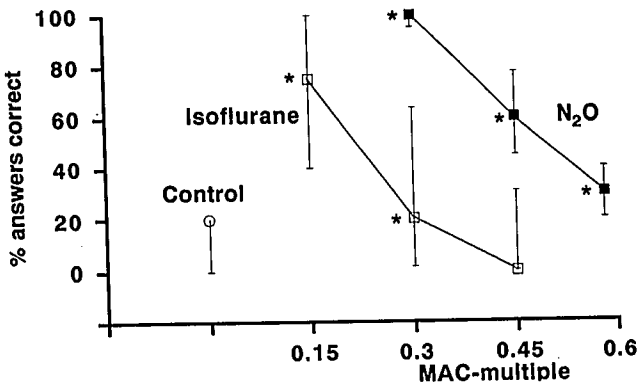

FIG. 3. Percentage of answers correct from each anesthetic concentration and from control (nonpresented) answers (medians; quartiles in parentheses). *$P < .05$ for percentage of answers correct compared to control questions.

preventing memory (estimated by probit analysis of the dose-response relationship) differed by 0.3 MAC (95% confidence intervals, 0.24–0.37) (see Appendix). The ED_{95} (concentration that prevented memory of 95% of answers) was 0.43 MAC isoflurane (95% confidence intervals, 0.36–0.55) and 0.73 MAC N_2O (95% confidence intervals, 0.65–0.87). The ED_{50} was 0.2 MAC for isoflurane (95% confidence intervals, 0.15–0.25) and 0.5 MAC for N_2O (95% confidence intervals, 0.43–0.55).

Memory of answers did not differ significantly between the early (75%, 40–90%) and late (80%, 60–80%) administrations of 0.15 MAC isoflurane. Memory of answers from the early administration of 0.3 MAC N_2O (100%, 95 to 100%) was significantly greater than from the later administration (80%, 20–80%; medians, quartiles; $P < 0.05$).

Spontaneous recall of answers was decreased by increasing anesthetic concentrations. Twenty percent of answers were spontaneously recalled from 0.15 MAC isoflurane (median 0, interquartile range, 0–20%), only one answer was spontaneously recalled from 0.3 MAC isoflurane, and spontaneous recall of all answers was prevented by 0.45 MAC isoflurane (fig. 4). Similarly, 0.3 MAC N_2O allowed spontaneous recall of 36% of answers, whereas only 10% were spontaneously recalled from 0.45 MAC N_2O (median, 0; interquartile range, 0–20%), and none from 0.6 MAC N_2O (fig. 5). The negative correlation between dose of anesthetic agent and prevention of spontaneous recall was statistically significant for both agents.

Conscious recognition of answers also was correlated negatively with anesthetic concentration of isoflurane and N_2O (figs. 4 and 5). Forty-two percent of answers were consciously recognized from 0.15 MAC isoflurane, 15% of answers from 0.3 MAC isoflurane (median, 0; interquartile range, 0–10%), and none from 0.45 MAC. One answer was consciously recognized from 0.6 MAC N_2O. Conscious memory of answers occurred only in subjects who had been able to respond purposefully to verbal command at a particular anesthetic concentration, *i.e.*, anesthetic concentrations that prevented purposeful response to command also abolished conscious memory.

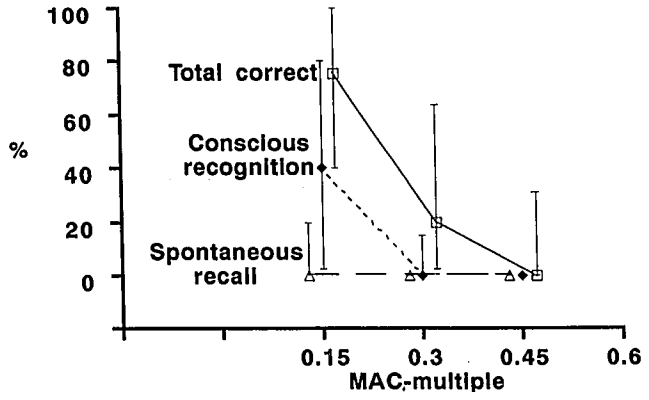

FIG. 4. For each isoflurane concentration, percentage of answers correct, consciously recognized, and spontaneously recalled (medians, quartiles).

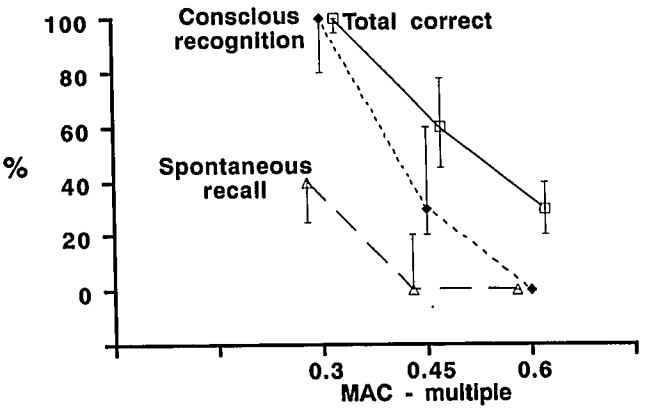

FIG. 5. For each N_2O concentration, percentage of answers correct, consciously recognized, and spontaneously recalled (medians, quartiles).

Unconscious memory occurred significantly at 0.15 and 0.3 MAC isoflurane (but not at 0.45 MAC) and at 0.45 and 0.6 MAC N_2O (fig. 6). Almost all answers from 0.3 MAC N_2O were consciously recognized, so that unconscious memory could not be tested.

Eight of 12 subjects presented with a behavioral message (nose or ear touching) at 0.45 MAC N_2O, consciously recalled the message although three of the eight did so only after being specifically cued by verbatim repetition of the introductory part of the message. No subject consciously recalled the message from 0.45 MAC isoflurane, but one guessed it correctly (see below). During the interview before the experiment, numbers of touches of directed and nondirected parts did not differ. Numbers of touches were greater in the follow-up interview at least

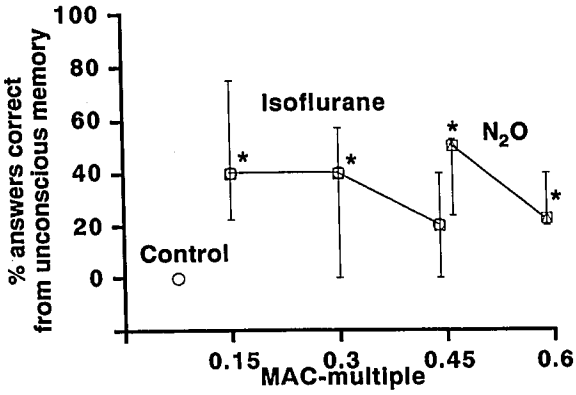

FIG. 6. For each anesthetic concentration, percentage of answers that were answered correctly from unconscious memory (without conscious recognition). *$P < .05$ compared to control questions (medians, quartiles).

partly because of its longer duration. The directed part (nose or ear) was touched significantly more often than the nondirected part in the follow-up interview after N_2O but not after isoflurane (table 1).

Discussion

Isoflurane and N_2O decrease all aspects of memory in a dose-related fashion. Statistically significant memory was allowed by all concentrations studied except 0.45 MAC isoflurane. Recognition memory was completely prevented by 0.45 MAC isoflurane and was greatly decreased (but not completely prevented) by 0.6 MAC N_2O. Unconscious memory was prevented by 0.45 MAC isoflurane but not by 0.6 MAC N_2O. These results are consistent with those of previous investigators.[31,35] Whether the arousing effect of surgery may increase the anesthetic concentrations required to prevent conscious and unconscious memory is not known.

Not all our data are consistent with data from other studies. The failure of 0.3 MAC N_2O to impair memory in our study contrasts with the finding by Robson *et al.* that 30–40% N_2O significantly impaired short-term memory of numbers[36]; with the finding of Parkhouse *et al.* that 30% N_2O impaired short- and long-term memory[37]; with the finding of Korttila *et al.* that 30% N_2O impaired free recall of words and performance in arithmetic tests[26]; with the effect of 30% N_2O on memory found by Ghoneim *et al.*[38]; and with the finding of Block *et al.* that 30% N_2O impaired conscious memory (with much less effect on tests of unconscious memory).[29] While Block *et al.* found evidence of unconscious (implicit) memory at 30% N_2O,[29] there was almost complete conscious memory of the material we presented at this concentration, so that with our methodology, we could not assess unconscious memory. These differences suggest that our memory task is more resistant to the amnesic effect of N_2O than the tasks used by previous investigators. We limited the number of questions used so as not to overload subjects with information, and we asked the questions before anesthetic administration to prime subjects to hear the answers. We used a multiple-choice format (with the correct answer to each question and four "distractors") to maximize the cue provided to the subject and to maximize memory, particularly unconscious memory.

TABLE 1. Effect of Behavioral Suggestions at 0.45 MAC Isoflurane and N_2O

	Isoflurane		N_2O	
	Before	After	Before	After
Directed part	0 (0–1)	2 (1–5)	1 (0–2)	3 (0.5–5)
Control part	0.5 (0–1)	1.5 (0–5)	0.5 (0–1)	1 (0–1.5)*

Numbers of touches of directed and control (nondirected) parts during the interviews before and after anesthetic administration (medians; quartiles in parentheses).

* $P < .05$ for difference between directed and nondirected behaviors in the postanesthetic interview.

Isoflurane in MAC-equivalent concentrations proved more potent than N_2O in diminishing memory (fig. 3). This is consistent with previous findings that isoflurane is more potent than MAC-equivalent concentrations of N_2O for sedation[39] and in suppression of auditory evoked responses[40] and peak velocity of saccadic eye movements,[41] and that other volatile anesthetics (*e.g.*, enflurane, halothane) are more potent than N_2O in impairing cognitive function and manual dexterity.[42,43]

Statistically significant memory existed from 0.6 MAC N_2O despite conscious recognition of only one answer by one subject (fig. 5). A significant percentage of answers presented at 0.15 and 0.3 MAC isoflurane and 0.45 and 0.6 MAC N_2O, which were not consciously recognized, were nevertheless answered correctly (fig. 6). These observations suggest that unconscious memory occurred at these anesthetic concentrations but was abolished by higher doses of isoflurane (0.45 MAC). We have not differentiated in this study between true unconscious (implicit) memory and "source amnesia" (meaning that patients consciously learned but later forgot).

Our value for MAC-awake for isoflurane is consistent with that recently determined by a similar technique[35] but is somewhat greater than the value of 0.25 MAC obtained when the inspired concentration of isoflurane was decreased in steps of 0.2–0.4%.[44] The value obtained in the latter study followed surgery and was obtained during elimination of the agent, when end-tidal partial pressure may be 25% less than arterial pressure.[45] For these reasons, we believe that our value, obtained after 15 min at a constant end-tidal concentration, is a more accurate measure of MAC-awake for isoflurane.

We found that MAC-awake as a fraction of MAC is lower for isoflurane (0.38) than for N_2O (0.64) (fig. 2). Similarly, MAC-awake determined by a technique similar to ours is 0.52 MAC for halothane and methoxyflurane and 0.6 MAC for ether,[46] indicating a difference between MAC-awake values for different agents. Thus, concentrations of different inhalational agents that are equivalent in terms of MAC (prevention of movement in response to surgical incision) may not be equipotent in achieving other anesthetic effects.

To calculate MAC-awake, we assumed that those subjects who responded voluntarily to command at the highest concentration would not have responded at the next step (0.6 MAC isoflurane and 0.75 MAC N_2O). We felt justified in making this assumption for two reasons: firstly, previous work indicates that 80% N_2O (0.76 MAC) prevents movement in response to verbal command in almost

all subjects;[31] secondly, work with other agents has shown that the dose-response curve for abolition of voluntary response to verbal command has a steep downward slope,[31,46] and our data show that 0.6 MAC N_2O and 0.45 MAC isoflurane are on the downward part of the dose-response curve. Nevertheless, it should be borne in mind that calculation of these values for MAC-awake rely on assumptions not tested.

Anesthetic concentrations that prevented voluntary response to command in an individual also abolished all conscious memory in that individual. The ED_{50} for prevention of memory was less than the ED_{50} for prevention of voluntary response to verbal command for isoflurane (0.20 MAC, 0.38 MAC) and N_2O (0.50 MAC, 0.64 MAC). Thus, anesthetic concentrations that prevent voluntary response also prevent conscious memory.

Values for ED_{50} for memory from our data apply only to this memory task, and different tasks may generate a different value for ED_{50}. We chose our task to mimic connected discourse, requiring cognition of language and grammatical sense. Thus, it is more relevant to prevention of awareness in the operating room than tasks requiring, for example, memory of single words.

The greater incidence of memory with N_2O may be due to its stimulatory effects. N_2O stimulates the sympathetic nervous system,[47–49] and injection of catecholamines has been shown to increase learning in rats during anesthesia.[50] Similarly, N_2O has some characteristics of a central nervous system stimulant.[27,29,51] It fails to supplement isoflurane-induced depression of cerebral metabolic rate[52] and may antagonize the electroencephalographic effects of volatile agents.‖

One subject provides anecdotal evidence for the enabling effect of arousal and catecholamines on memory. While receiving N_2O during his first session, he experienced dysphoria and for the next 3 days suffered recurrent illusions that he was still receiving the anesthetic agent ("flashbacks"). He was apprehensive before receiving isoflurane 10 days later. His systolic blood pressure increased to 180 mmHg while breathing oxygen before the experiment began, and isoflurane was withheld for 20 min until his blood pressure returned to normal levels. The study was subsequently uneventful. During the post-study interview, he answered 17 of 20 questions correctly, the best performance of any subject in the isoflurane part of the experiment. He received a message during isoflurane administration to touch his nose, and touched his nose six times during the post-study interview, compared to no nose touches during the pre-study interview. Near the end of the follow-up interview, when cued with the introductory part of the behavioral message, he guessed "Maybe touch my nose"; he was the only subject to guess the message correctly after isoflurane administration. Was the increased learning shown by this subject related to his increased level of central nervous system arousal and circulating catecholamines? If this were so, it would suggest that learning can occur during anesthesia in cases with high levels of central nervous system or sympathetic nervous system arousal.[50]

The emetic effect of N_2O has been reported previously,[23] and subjects who receive N_2O as part of their anesthetic may have a greater incidence of postoperative vomiting.[53] Although the incidence of vomiting did not differ between 0.45 MAC isoflurane and 0.45 MAC N_2O, vomiting was more common with N_2O than with isoflurane in concentrations equipotent in preventing response or memory.

Vomiting during N_2O administration was episodic rather than continuous. When vomiting occurred, the mouth piece was removed for periods of 1–2 min. N_2O administration in these subjects was subsequently prolonged by 5–10 min, in proportion to the duration of breathing room air. The rate of washout of N_2O (from either lungs or brain) while subjects breathe room air depends on pulmonary and cerebral time constants, which are identical for washin and washout. Because we prolonged N_2O administration by a factor of five times the duration of breathing room air, we assume we overcame the effects of eliminating the agent during vomiting.

The prolonged time to full recovery after N_2O (7 hr *vs.* 2 hr for isoflurane) was due principally to the residual nausea felt by many subjects after N_2O. There was no evidence of tolerance developing when comparing the effects of the early and late administrations of the lowest concentration of each agent on memory.

The behavior directed during isoflurane administration (to touch the ear or nose) did not occur more commonly than the control behavior during the follow-up interview. The behavior directed during N_2O administration was exhibited significantly more often than the control behavior, but because of the 66% incidence of either spontaneous or cued recall of the suggestion, this may reflect a mix of conscious and unconscious memory formed during N_2O administration. Our finding for isoflurane differs from previous findings of a positive effect of an intraoperative message to touch a body part[9,10,54] but is consistent with other studies that failed to find an effect.[16,17]

A number of factors in the design of the study should consider when interpreting our results. The question task we used differs from tasks used in other studies of implicit memory[10,55] but has been used previously to demonstrate unconscious memory during anesthesia.[56] We defined unconscious memory as occurring when answers not con-

‖ Smith NT, Hoff BH, Rampil IJ, Sasse FJ, Flemming DC: Does thiopental or N2O disrupt the EEG during enflurane? (abstract) ANESTHESIOLOGY 51:S5, 1979.

sciously recognized as having been presented were correct more often than control answers. This definition is not generally used for unconscious memory because of the difficulty in distinguishing "source amnesia" (see above). However, we believe the definition we used is the most relevant for the clinician who wished to prevent conscious memory of events during anesthesia.

We used the same order of administration of different concentrations in all subjects. This could have concealed effects of either duration of administration or of preceding concentrations on results at a given concentration. Although the order of administration of different concentrations of agent might have been rotated, we chose the simpler approach, considering that any bias due to order would apply equally to both anesthetics and thus not affect interagent comparisons. There was no significant difference between memory at the early and late administrations of isoflurane, suggesting that rotating the concentrations would not have altered the results. The difference between results at the early and late administrations of 0.3 MAC N_2O we considered to be probably due to vomiting at 0.6 MAC N_2O, so that rotating the concentrations would have confounded the dose-response relationship at subsequent concentrations of N_2O.

A larger number of test and control questions would have been useful; unfortunately, we were limited in the amount of material we could present to subjects in each session. We believed too many questions would lead to information overload (*i.e.*, exceed the capacity to remember, decrease attention to individual questions, and decrease curiosity about the questions). In our desire to maximize learning of what was presented, we limited the number of questions presented preoperatively and the number answered at each concentration. We used eight fixed sets of five questions. The sets of questions were not precisely equated in difficulty; there were small and perhaps inevitable differences between questions and groups of questions. This effect was allowed for by rotating questions among different concentrations and agents and by rotating the control questions among all groups of questions and all concentrations of both agents. Because of the limits in the numbers of subjects we studied, complete counterbalancing was not possible, and each set of questions was administered at a particular concentration and was used as the control set of questions either one or two times. At each anesthetic concentration, one or two subjects heard no answers.

We did not use a placebo control group (*i.e.*, subjects who did not receive an anesthetic agent). However, the effect of placebo is evident in the responses of the subjects given 0.3 MAC N_2O; subjects had memory for almost all answers provided at this concentration. Additionally, in a previous study we presented answers to these questions before anesthesia and surgery (*i.e.*, a placebo control group). Memory of the answers presented was tested 24 hr later and was found to be 97%.[17]

Earlier testing of our subjects might have increased memory of the answers presented. We delayed testing our subjects for 24 hr because we considered that sequential testing (*i.e.*, conducting more than one postanesthesia test on a given subject) would not provide independent data. The answers given initially would be ingrained and the likelihood of their repetition increased. Thus we believed we had but one opportunity to evaluate the effect of what we had done (*i.e.*, what the subject had learned). We chose the 24-hr point because implicit learning may become stronger with some durations,[57] but more importantly, because a 24-hr period might be more likely to assure elimination of residual depressant or noxious effects (*e.g.*, nausea). The persistence of unconscious memory over a 24-hr period has been demonstrated previously.[29]

Assessment of ear and nose touching was made by a number of observers, and we have no data on the reliability of this observation. However, we believe a dedicated observer to be able to count these behaviors reliably. Subjects were unaware that this observation was being made, and ear and nose touches were counted by an observer who had no other duties and who was unaware of which behavioral message the subject had received.

In summary, isoflurane was more potent than N_2O in MAC-equivalent doses in decreasing conscious and unconscious memory and voluntary response to verbal command. Memory (conscious and unconscious) was decreased by both agents in a dose-related fashion and was prevented by 0.45 MAC isoflurane but not by 0.6 MAC N_2O.

Appendix

This Appendix describes the method of statistical analysis used to interpret the relationship between dose of anesthetic agent and suppression of memory of answers to questions.

Methods

Generalized linear models for the probability P of correct response were fitted using the statistical analysis system procedure probit.# The general form of model used was $P = C + (1 - C)F(L)$, where C is a baseline value to be estimated, F is a specified cumulative distribution function (either logistic or normal) and $L = \Sigma\, bj\, xj$ is the value of linear model for the effects of dose, type of anesthetic (or control), and subject. The basic model specified dose-response curves for the two anesthetics that were parallel and linear on the transformed (L) scale. It included the following covariates: 1) anesthetic dose (MAC-multiples), 2) an indicator variable for the anesthetic agent (coded 1 if the

SAS/STAT Users Guide, Version 6. 4th edition. Cary, NC, SAS Institute Inc., 1990, p 1325.

observation pertained to that agent and 0 if it did not), 3) an indicator variable for control data, and 4) regressor variables corresponding to 11 of the 12 subjects. Subject effects were coded as deviations from an unweighted mean over subjects. Generalizations of this model were used to test for nonparallelism of the two dose-response curves and for nonlinearity of the dose-response curves for each anesthetic agent. An arbitrary value of dose was assigned to the control data to allow their inclusion in the dose-response model, with the value assigned having no effect on the dose-response relationship of the model.

Using fitted results from the basic model, estimates and 95% fiducial confidence limits[58] were computed for 1) the difference between doses of the two agents that produced the same effect (suppression of memory) and 2) the doses of each agent that allowed memory 5% and 50% above baseline values. Conceptually, these are the doses of anesthetic preventing memory of almost all of (ED_{95}) and 50% of (ED_{50}) the information presented. These calculations utilized the estimated parameter values and estimated variances and covariances for dose and anesthetic effects. Subject effects were ignored, so that values for ED_{95} and ED_{50} correspond to an unweighted mean over the 12 subjects.

Results

For the basic model, the negative log-likelihood was 168.6 for the logistic distribution and 169.2 for the normal distribution (smaller value indicating better fit to the data). Neither tests for nonparallelism nor tests for nonlinearity of the dose-response relationships of the two agents achieved statistical significance, suggesting that dose-response relationships were linear for both agents and parallel to each other.

For the basic model, assuming parallel dose-response curves for the two anesthetics and linear dose-response relationships on the transformed scale, the baseline rate of correct answers was 10.2 (3.4%) for the logistic distribution and 9.9 (3.4%) for the normal distribution (estimated values, SE). This was almost identical to the observed rate of 10.2 (3.2%).

The difference between doses of N_2O and isoflurane (as MAC-multiples) that suppressed memory equally was 0.30 MAC (0.24–0.37) (predicted value, 95% confidence limits) for both logistic and normal distributions. The doses of each agent leading to expected response levels 5% and 50% above baseline (*i.e.*, ED_{95} and ED_{50}) were greater for N_2O than for isoflurane (table A1). Results were similar using both the normal and the logistic transformation distribution models (table A1).

TABLE A1. ED_{50} and ED_{95} for Suppression of Memory by Isoflurane and N_2O

Anesthetic	Distribution	ED_{95}	ED_{50}
Isoflurane	Logistic	0.43 (0.36–0.57)	0.20 (0.15–0.25)
	Normal	0.43 (0.36–0.55)	0.20 (0.15–0.25)
N_2O	Logistic	0.73 (0.65–0.89)	0.50 (0.46–0.55)
	Normal	0.73 (0.65–0.87)	0.50 (0.43–0.55)

MAC multiples of isoflurane and N_2O causing 95% and 50% suppression of memory (ED_{95} and ED_{50}), calculated from logistic and normal distributions of the data (estimated values; 95% confidence intervals in parentheses).

Discussion

Two alternate formulations of the dose-response models were considered: logistic transformation models assuming random subject effects (random as opposed to fixed intercepts for each subject) and conditional logistic regression models conditioning on subjects (as opposed to the unconditional logistic regression modelling of subject effects used here). However, available implementations of these alternative models such as EGRET¶ did not allow for a non-zero baseline rate, which seemed of compelling need for these data. Conditional and unconditional logistic regression excluding the control data were compared assuming a zero baseline; results were quite similar, suggesting that the bias in parameter estimates associated with unconditional logistic regression adjustment for stratum (subject) effects[59] was minor for these data.

Data from both control and presented questions were used to derive the baseline rate for answering questions correctly. To examine the consistency of baseline rates estimated from these two sources, the baseline rate also was estimated without the control data, giving an estimate of the baseline rate (SE) of 10.2 (7.1%) for the logistic distribution and 9.0 (7.0%) for the normal. These were in remarkably good agreement with the observed rate of 10.2% from the control data and with estimates of the baseline rate derived while including the data for the control questions. As the above results show, inclusion of the control data in estimation of the baseline rate reduced its estimated standard error by more than 50%. This resulted in substantially narrower confidence intervals for the estimated doses of the two agents, corresponding to an expected response 5% above the baseline level.

The assumption that the dose of anesthetic and the anesthetic agent used have a common effect across subjects is unrealistic, and therefore, confidence intervals are probably narrower than they should be. The assumption that all questions are equally likely to be answered correctly also is unrealistic, with similar effect on the confidence intervals. However, models that do not require these assumptions seemed too complex for the small amount of data we had available. The estimates of ED_{50} and ED_{95} for memory suppression for the two agents represent average effects obtained from models that appear to satisfactorily reflect the main features of the data.

The data presented were not normally distributed; thus, in the main section of the paper, between-group comparisons were performed using Wilcoxon's matched pair test. In estimation of dose-response curves in this Appendix, the non-normal distribution of the data was taken into account in the selection of the parametric analyses that were applied.

¶ EGRET Reference Manual. First draft. Seattle, Washington, Statistics and Epidemiology Research Corp., 1990.

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