

# Long-term Impairment of Acquisition of a Spatial Memory Task following Isoflurane–Nitrous Oxide Anesthesia in Rats

Deborah J. Culley, M.D.,\* Mark G. Baxter, Ph.D.,† Rustam Yukhananov, M.D., Ph.D.,\* Gregory Crosby, M.D.‡

**Background:** The authors demonstrated previously that isoflurane–nitrous oxide anesthesia attenuates performance improvement on an already-learned spatial memory task and that the effect persists for weeks. This experiment was designed to test the hypothesis that learning of new information is particularly susceptible to prolonged disruption after general anesthesia.

**Methods:** Six- (n = 5) and 20- (n = 5) month-old male Fischer 344 rats were anesthetized for 2 h with 1.2% isoflurane, 70% nitrous oxide, and 30% oxygen. Age-matched control rats received 30% oxygen and 70% nitrogen (n = 5 per group). Rats breathed spontaneously, and anesthetic and oxygen concentrations were measured. Spatial learning was assessed daily for 21 days on a 12-arm radial maze (RAM) beginning 48 h after anesthesia. In a *post hoc* experiment to examine locomotion, swim speed was assessed in a separate group of identically treated rats (n = 3 per group) for 4 days beginning 48 h after anesthesia.

**Results:** Aged rats were slower to complete the maze, made fewer correct choices before first error, and made more errors at baseline than young rats ( $P < 0.05$ ). Anesthesia worsened maze performance in both age groups, as evidenced by increased time to complete the maze and a decreased number of correct choices before first error ( $P < 0.05$ ), but there were no statistically significant differences in total number of errors. Interestingly, there were no age-by-anesthesia interactions. Aged rats swam slower than adult rats ( $P < 0.001$ ), but there were no differences between the control and anesthesia groups.

**Conclusions:** Isoflurane–nitrous oxide anesthesia is associated with a persistent deficit in RAM performance that is not explained by impaired locomotion. This impairment occurs in adult and aged rats, indicating that it is not an age-specific phenomenon. Thus, RAM performance is altered after general anesthesia for longer than predicted by the pharmacology of the drugs used, which, by inference, suggests a long-term deficit in learning/memory.

COGNITIVE dysfunction is common immediately after surgery and general anesthesia. Although recovery is usually rapid, some patients report lingering difficulty with concentration and memory for days or weeks after surgery. In fact, recent clinical studies indicate that such cognitive impairment persists for at least a week in middle-aged patients<sup>1</sup> and for months in a substantial percentage of the elderly.<sup>2</sup> Whether this is a result of the effects of surgery, general anesthesia, or other perioperative confounders remains unclear.

In a previous study in rats, we investigated the possibility that general anesthesia itself could contribute to persistent learning impairment. In particular, we demonstrated that an uncomplicated isoflurane–nitrous oxide anesthetic attenuates improvement on an already-learned spatial memory task for weeks in aged but not young rats.<sup>3</sup> In that study, the young rats, despite having received identical training, mastered the task sooner and therefore were more familiar with it at the time of anesthesia than the aged rats. Thus, at the time of anesthesia, the groups differed in both age and task mastery, which limited our ability to define the relative contribution of each to the postanesthesia performance impairment we observed. Because we hypothesized that the ability to learn something new may be especially susceptible to prolonged disruption after general anesthesia, we designed this experiment to evaluate the influence of previous general anesthesia on subsequent acquisition of spatial memory.

## Materials and Methods

This protocol was approved by the Harvard Medical Area Standing Committee on Animals and by the Harvard University Faculty of Arts and Sciences Standing Committee on the Use of Animals in Research and Teaching. Six- and 20-month-old Fischer 344 rats were acquired from the National Institute on Aging colony at Harlan (Harlan Sprague-Dawley, Inc., Indianapolis, IN). After a 1-week acclimation period in the laboratory, rats were food restricted and habituated to a 12-arm radial maze (RAM) for 10 min daily over 3 days. During this interval, the rat was able to explore the maze in which food rewards (quarter pieces of Froot Loops cereal [Kellogg's, Battle Creek, MI]) were scattered randomly. The purpose of habituation is to acclimate the animal to the unfamiliar environment of the maze; because food is distributed randomly, habituation does not directly assist subsequent maze learning. Thereafter, rats were ran-

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

\* Instructor, ‡ Associate Professor, Department of Anesthesia, Harvard Medical School, Brigham & Women's Hospital, Boston, Massachusetts; and the Department of Psychology, Harvard University, Cambridge, Massachusetts. Submitted for publication January 6, 2003. Accepted for publication September 24, 2003. Supported by a Jahnigen Award from the American Geriatrics Society, New York, New York, and a Harvard/Hartford Foundation New Investigator Award, Boston, Massachusetts (to Dr. Culley), and by National Institute of Aging grant No. R01 AG20253, Bethesda, Maryland (to Dr. Crosby). This work was presented in abstract form at the Annual Meetings of the American Society of Anesthesiologists, Orlando, Florida, October 12–16, 2002; and at the Annual Meeting of the American Geriatrics Society, Washington, D.C., May 8–12, 2002.

Received from the Department of Anesthesia, Harvard Medical School, Brigham & Women's Hospital, Boston, Massachusetts; and the Department of Psychology, Harvard University, Cambridge, Massachusetts. Submitted for publication January 6, 2003. Accepted for publication September 24, 2003. Supported by a Jahnigen Award from the American Geriatrics Society, New York, New York, and a Harvard/Hartford Foundation New Investigator Award, Boston, Massachusetts (to Dr. Culley), and by National Institute of Aging grant No. R01 AG20253, Bethesda, Maryland (to Dr. Crosby). This work was presented in abstract form at the Annual Meetings of the American Society of Anesthesiologists, Orlando, Florida, October 12–16, 2002; and at the Annual Meeting of the American Geriatrics Society, Washington, D.C., May 8–12, 2002.

Address reprint requests to Dr. Crosby: Department of Anesthesiology, Brigham & Women's Hospital, 75 Francis Street, Boston, MA 02115. Address electronic mail to: g.crosby@zeus.bwh.harvard.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

domly assigned to anesthesia or control groups. Rats randomized to the anesthesia group (five 6-month-old and five 20-month-old rats) received 1.2% isoflurane in 70% nitrous oxide/30% oxygen for 2 h in an anesthetizing chamber, whereas the control group (five 6-month-old and four 20-month-old rats) received air/oxygen ( $F_{IO_2}$  0.3) at identical flow rates for 2 h in identical chambers. Anesthetic and oxygen concentrations were measured continuously (Datex, Tewksbury, MA; Ohmeda, Madison, WI), and the temperature of the anesthetizing chamber was controlled to maintain rat body temperature at  $37 \pm 0.5^\circ\text{C}$ . Discontinuing the anesthetics, administering 100% oxygen for 5 min, and subsequent removal of the rat from the anesthetizing chamber terminated treatment. Rats recovered for 48 h so as to avoid the confounding influence of residual anesthetic before 21 days of RAM testing.

The RAM tests spatial working and reference memory and assesses the integrity of the frontal cortex, perirhinal/entorhinal cortex, and hippocampus.<sup>4-6</sup> In addition, it can detect subtle differences in learning and memory caused by aging, sedative medications, and anesthetics.<sup>3,7,8</sup> The maze consists of a central platform that communicates with 12 arms, each of which is baited with a hidden food reward. The walls of the maze display simple geometric designs providing fixed extramaze cues to assist spatial navigation. To ensure motivated performance, rats were food restricted to 85% of free-feeding body weight but were given free access to water in the home cage.

Testing consisted of a daily 15-min session in which the rat was placed on the central platform of the maze with all arms baited. The rat was allowed to choose arms in any order until all 12 arms were visited or 15 min elapsed. A correct choice was defined as one in which the rat entered a baited arm not previously explored, whereas an error was scored when the rat entered an arm it had previously visited or failed to enter the arm in 15 min. The number of training trials required to meet standardized performance criteria, defined as 11 correct choices with one or fewer errors in less than 15 min for 2 consecutive days, was recorded. In addition, the number of correct choices before first error, number of errors, and time to complete the maze were measured for each trial. Trial results were grouped and analyzed in 3-day blocks.

A *post hoc* experiment was performed to exclude motor impairment as a cause of altered postanesthetic performance. Separate young and aged rats were randomly assigned to a control or anesthesia group ( $n = 3$  per group) as described previously, and swim speed was tested in a Morris Water maze for 4 days beginning 48 h after anesthesia. The maze consists of a circular 180-cm tank filled with water heated to  $27^\circ\text{C}$  and, to make the water opaque, a nontoxic white watercolor paint. The object is for the rat to locate a Plexiglas escape platform

that extends from the floor of the tank to approximately 1.5 cm below the surface of the water. Black curtains affixed with four high-contrast black and white visual cues positioned at the north, east, south, and west sides surround the tank. Activity is taped using a small video camera located above the center of the maze and connected to a tracking system and videocassette recorder. Each animal's swim speed was collected using HVS Water for Windows (HVS Image, Hampton, UK). For each trial, the rat was placed in the pool along the perimeter of the tank, facing the tank wall, with the four entry points (north, east, south, and west) randomized across trials, but the same sequence of start points was used for each rat. The trial ended when the rat located the escape platform or when 90 s had elapsed, at which time the rat was guided to the platform. In either case, the rat was allowed to remain on the platform for 15 s before being returned to a holding cage for a 30-s intertrial interval. Average swim speed (cm/s) per day was recorded and analyzed.

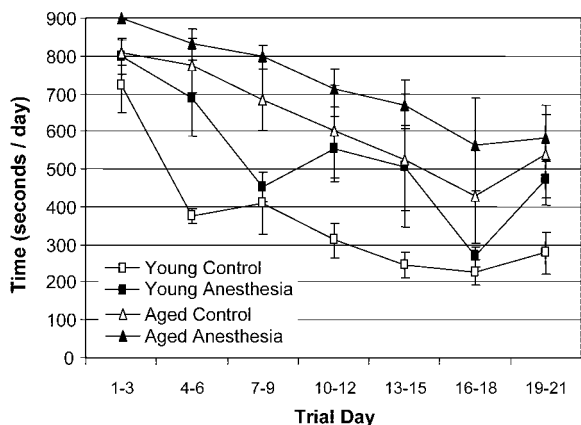
#### Statistics

Performance on the RAM and Morris water mazes was analyzed by repeated-measures ANOVA, with anesthesia group and age as between-subject factors and day of testing as the within-subject factor. All analyses were performed in SYSTAT 7.0 for Windows (SPSS Inc., Evanston, IL).

#### Results

During acclimation to the RAM before anesthesia, one of the animals previously randomized to the aged control group fell off the maze and was excluded from the study. For the remainder, there was a main effect of training trial on the number of correct choices to first error, errors, and time to complete the maze, indicating a learning effect in both age groups ( $P \leq 0.001$ ; figs. 1-3). There was also a main effect of age on time to complete the maze, number of correct choices to first error, and error rate ( $P \leq 0.001$ ; figs. 1-3). Thus, aged rats took longer to complete the maze, made fewer correct choices before first error, and made more errors than young rats.

Concerning the effect of anesthesia, days to standardized performance criteria were unavailing because too few animals satisfied the criteria during the 3-week testing interval such that the data failed the equal variance test. In contrast, previously anesthetized rats were made worse by previous anesthesia as measured by time to complete the maze and number of correct choices before first error ( $P \leq 0.05$ ; figs. 1 and 2, respectively). There was, however, no effect of anesthesia on number of errors (fig. 3) and no statistically significant interaction between age and anesthesia condition for any of the parameters. Swim speed data revealed a main effect of

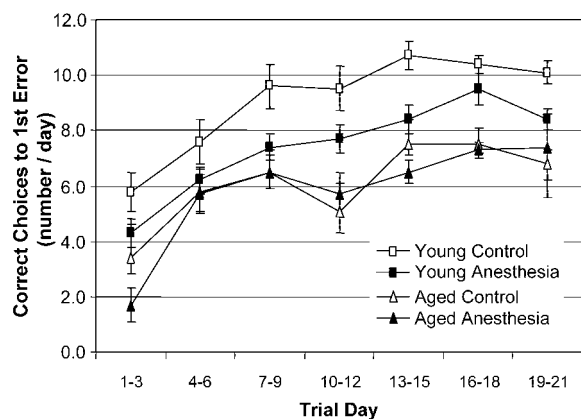


**Fig. 1.** Time to complete the maze. Time data from the 12-arm radial maze were grouped into 3-day blocks and analyzed with a repeated-measures ANOVA, with anesthesia group and age as between-subject factors and day of testing as the within-subject factor. There was a main effect of age ( $P \leq 0.001$ ), training trial ( $P \leq 0.001$ ), and anesthesia ( $P \leq 0.05$ ), demonstrating that aged rats performed worse than young rats, that there was a learning effect, and that previously anesthetized rats performed worse than control rats. Data expressed as mean  $\pm$  SEM.

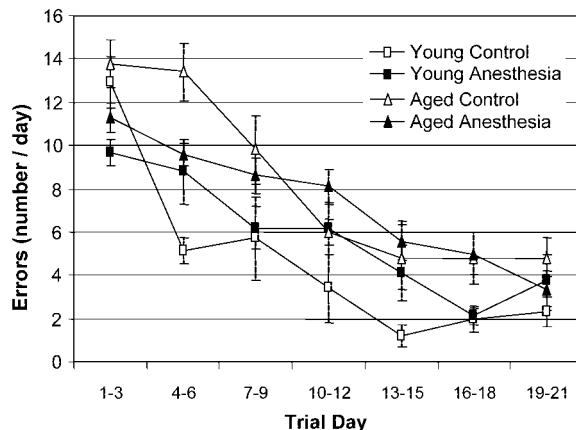
age ( $P < 0.001$ ) and trial day ( $P < 0.001$ ), indicating a practice or learning effect. There were, however, no main effects of anesthesia condition ( $P > 0.05$ ), indicating intact locomotion in the anesthetized rats (fig. 4).

**Discussion**

The principal finding of this study is that a single isoflurane-nitrous oxide general anesthetic produces lasting impairment in the ability of both adult and aged rats to acquire and perform a spatial memory task. Previous isoflurane-nitrous oxide anesthesia produced an



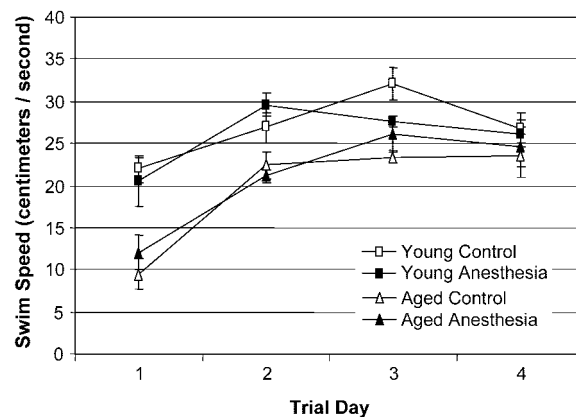
**Fig. 2.** Number of correct choices to first error. Data were grouped into 3-day blocks and analyzed with a repeated-measures ANOVA, with anesthesia group and age as between-subject factors and day of testing as the within-subject factor. There was a main effect of age ( $P \leq 0.001$ ), training trial ( $P \leq 0.001$ ), and anesthesia ( $P \leq 0.05$ ), demonstrating that aged rats performed worse than young rats, that there was a learning effect, and that previously anesthetized rats performed worse than control rats. Data expressed as mean  $\pm$  SEM.



**Fig. 3.** Number of errors. Data were grouped into 3-day blocks and analyzed with a repeated-measures ANOVA, with anesthesia group and age as between-subject factors and day of testing as the within-subject factor. There was a main effect of age ( $P \leq 0.001$ ) and training trial ( $P \leq 0.001$ ), demonstrating that aged rats performed worse than young rats and that there was a learning effect. There was no effect of previous anesthesia on number of errors ( $P \geq 0.05$ ). Data are expressed as mean  $\pm$  SEM.

increase in the time required to complete the RAM and a decrease in the number of correct choices before first error. It is unlikely that this impairment is caused by residual anesthetic, because nearly all isoflurane is cleared from the brain within minutes after a 2-h anesthetic,<sup>9</sup> with nitrous oxide being cleared even faster, and we did not begin behavioral testing until 48 h after anesthesia. Therefore, in rats, a general anesthetic with isoflurane-nitrous oxide impairs performance on a spatial memory task for considerably longer than the pharmacokinetics of the drugs would predict.

The RAM is a standard and well-validated test of spatial learning and memory, but noncognitive variables such as



**Fig. 4.** Swim speed. Swim speed was tested in a Morris water maze, and the average speed per trial day was analyzed with a repeated-measures ANOVA, with anesthesia group and age as between-subject factors and day of testing as the within-subject factor. There was a main effect of age ( $P \leq 0.001$ ) and training trial ( $P \leq 0.001$ ), demonstrating that aged rats swam slower than young rats and that there was a learning effect. However, there was no effect of previous anesthesia on swim speed ( $P \geq 0.05$ ), indicating that locomotion is intact in previously anesthetized rats. Data are expressed as mean  $\pm$  SEM.

desire to eat and ability to walk can influence it, so conclusions must be drawn cautiously. In our experiment, insufficient desire to eat is unlikely to have been a problem given that each rat received the same daily food allotment during the period of food restriction, consumed it avidly within minutes, and maintained stable weight. This agrees with data showing that body weight is stable after isoflurane anesthesia in Fischer 344 rats.<sup>10</sup> Likewise, as demonstrated by the similar swim speeds in control and anesthetized rats, there is no persistent post-anesthetic locomotor impairment to account for poor performance on the RAM. Stress such as might occur from the new surroundings of the anesthetic chamber or smelling the anesthetic vapor is also unlikely to have influenced the results. General anesthesia tends to reduce endocrine markers of stress, and if the anesthetic chamber is stressful, the controls should be most affected because they spent the most time in it awake. Moreover, for stress to affect learning behavior adversely and persistently, it must be chronic and sustained, which is certainly not the case here. In rats subjected to 15–30 min of restraint, swim, or footshock stress, for example, behavioral differences are detectable on the open field test 24 h but not 48 h later.<sup>11</sup> Finally, impaired smell or poor health can be excluded, because rats use extramaze cues rather than smell to navigate the maze<sup>12</sup> and even aged rats suffer no apparent ill effects in the first month after general anesthesia.<sup>3</sup> Thus, given that abnormalities in general health, appetite, stress, and locomotion are unlikely to account for our results, we infer that the most logical explanation for the behavioral impairment we observed is a long-term change in the ability to learn a spatial memory task. To the extent that performance on the RAM is mediated by the hippocampal memory system, including associated cortical areas, the results also suggest that isoflurane–nitrous oxide anesthesia affects the hippocampal spatial memory apparatus for several weeks in rats.

These results expand on a previous study from this laboratory demonstrating that spatial learning is impaired for at least 3 weeks after general anesthesia in aged rats but that it improves spatial learning (relative to their baseline) in young rats.<sup>3</sup> Differences in study design probably account for the disagreement between our earlier and current studies. The earlier study was concerned mainly with established or consolidated memory, because rats were trained on the maze daily for 2 months before anesthesia and had largely learned it. In contrast, here, we examined the acquisition of new spatial memory, because the first maze trial occurred 48 h after anesthesia. This distinction is likely to be important, because the neurobiologic processes underlying memory acquisition and consolidation differ.<sup>13</sup> In particular, presumably because the neurochemical and structural modifications required for consolidation have yet to develop, newly acquired memory is more fragile. Thus, in

our previous study, the fact that the young rats learned faster and were more familiar with the task at the time of anesthesia than the aged rats implies that their memory for the maze was better consolidated at the time of anesthesia than that of the old rats. This was the impetus for the present experiment, because it suggested that ability to acquire and act on new information might be more susceptible to prolonged disruption after general anesthesia than ability to recall or relearn a previously familiar task. The current results support this hypothesis, because acquisition of a new spatial task was impaired in both the young and aged rats.

Recovery from general anesthesia is traditionally regarded as being rapid and complete, but our results suggest otherwise. They are also not the first in this regard. Previous work shows that isoflurane-induced burst suppression is more effective and longer lasting than electroconvulsive therapy for the treatment of refractory depression,<sup>14</sup> suggesting that persistent changes in brain function result from the treatment. There is also evidence, primarily in developing brain, that common anesthetics produce lasting structural and functional changes in the central nervous system. During the perinatal period, halothane anesthesia leads to learning deficits and delayed behavioral development in rats<sup>15</sup> and isoflurane and nitrous oxide produce widespread apoptosis, abnormalities in hippocampal synaptic function, and a learning deficit that lasts at least 4 months.<sup>16,17</sup> Although the relevance of such effects to the adult brain is unclear, nitrous oxide and ketamine, both of which block excitatory neurotransmission at *N*-methyl-D-aspartate receptors, produce a distinctive neurotoxic reaction in the cerebral cortex of adult rats at concentrations within the range used for human anesthesia.<sup>18,19</sup> Furthermore, the toxic reaction is greater in aged than young rats.<sup>20</sup> Additional evidence for a prolonged effect of general anesthetics or analgesics includes observations that a single dose of morphine produces acute tolerance and rapid but persistent changes in neural gene expression<sup>21</sup> and that multiple anesthetics with pentobarbital reduce central cholinergic binding for at least 2 months.<sup>22</sup> Taken together with this and our previous study,<sup>3</sup> these reports suggest that recovery of central nervous system function after general anesthesia may not be as rapid and complete as previously thought.

This study is limited in a few important ways. First, our conclusion that the performance deficit reflects cognitive impairment is an inference from the behavior and not a direct measure of learning. Such an inference seems sound and reasonable, however, inasmuch as for reasons given earlier, several potential confounders are unlikely to have materially influenced the results. Second, we did not measure physiologic variables such as arterial blood pressure and blood gases in the experimental animals. This is because an invasive procedure such as cannulation of a vessel would introduce the

confounder of surgery and perhaps limit mobility. Furthermore, we have already established that this anesthetic produces minimal physiologic perturbations in Fischer 344 rats of the ages used here.<sup>3</sup> Thus, in previous experiments using identical anesthetic dosages and procedures, we have shown that changes in arterial blood pressure and blood gases are minor and that values remain well within the normal range in both young and aged rats.<sup>3</sup> Accordingly, although it is not possible to exclude abnormal physiology associated with general anesthesia definitively as an explanation for the sustained learning impairment, it is unlikely to be responsible. Supporting this position, studies in humans fail to show an association between intraoperative hypotension or hypoxemia and postoperative cognitive dysfunction.<sup>1,2</sup> Third, we studied a combination of anesthetic agents at a single dose. Hence, it is impossible to say whether the effect is dose dependent, is caused by one or both of the agents used, or is nonspecifically related to loss of consciousness. Additional experiments are necessary to address these issues. Given the protective effects of isoflurane in some models of cerebral injury<sup>23</sup> and studies showing that new learning is particularly sensitive to *N*-methyl-D-aspartate receptor antagonists,<sup>24</sup> it is tempting to speculate that nitrous oxide might be the offending agent. However, both isoflurane and nitrous oxide have been shown to produce persistent abnormalities in hippocampal synaptic neurotransmission and learning under some conditions.<sup>16,17</sup> Fourth, we did not correct for age-related decreases in minimal alveolar concentration. The fact that both young and aged rats were impaired on the task indicates, however, that a difference in anesthetic depth between the groups does not explain the results nor does it exclude the possibility that "lighter" anesthesia would be devoid of such behavioral impairment.

Memory is a complex process involving multiple brain regions, neurotransmitter systems, intracellular signaling molecules, and genes.<sup>13,25</sup> General anesthetic agents have known effects on many of these neurochemical events,<sup>26</sup> but it is difficult to speculate how they may influence memory acquisition over the longer term. However, a few points are noteworthy. First, activation of *N*-methyl-D-aspartate glutamate receptors is required for long-term memory formation,<sup>27,28</sup> and  $\gamma$ -aminobutyric acid receptors provide major inhibitory influences on memory processing.<sup>28</sup> These neurotransmitter systems are well represented in the hippocampus, and general anesthetics, including isoflurane and nitrous oxide, act on them.<sup>26</sup> Second, memory consolidation has several temporal phases lasting hours to days,<sup>13,25</sup> with changes in synaptic efficiency as well as synaptic remodeling and growth of new synaptic connections ultimately being required.<sup>13,27,29,30</sup> Dysfunction in any or all of these events could contribute to cognitive deficits that linger long after general anesthesia, but further study is

needed to understand the nature of the underlying neurobiologic impairment.

There has been much recent interest in human postoperative cognitive dysfunction. This has been fueled largely by prospective clinical studies showing that general anesthesia and surgery are associated with long-lasting cognitive impairment in 10–14% of elderly patients<sup>2</sup> and that a large percentage of middle-aged and young patients suffer similarly for 1 to 2 weeks after surgery.<sup>1</sup> Several theories have been advanced to explain these observations, but although there is general agreement that the problem is likely to be multifactorial, the causes have not been established. Because of important differences between rats and humans in how and where memories are stored in the brain and how learning is tested, the relevance of our results to the human situation is unclear. The fact that isoflurane-nitrous oxide anesthesia is associated with long-lasting impairment in the ability of rats to refine established spatial memory<sup>3</sup> or acquire a new spatial task suggests, however, that the role of general anesthesia in postoperative cognitive dysfunction should be examined further.

We conclude, therefore, that general anesthesia with isoflurane-nitrous oxide produces lasting impairment in the ability of rats to acquire and perform a spatial memory task. This impairment occurred in both young and aged animals, indicating that it is not an age-specific phenomenon, but whether it is caused by the anesthetized state or the drugs used to achieve it cannot be determined. Because it is not easily explained by stress, abnormalities in locomotion, or the pharmacokinetics of the drugs involved, we infer that the performance impairment represents a persistent anesthesia-induced change in neural structures or biochemical cascades mediating learning and memory. If this is true, the results may have implications for understanding prolonged postoperative cognitive dysfunction in humans.

## References

1. Johnson T, Monk T, Rasmussen LS, Abildstrom H, Houx P, Korttila K, Kuipers HM, Hanning CD, Siersma VD, Kristensen D, Canet J, Ibanaz MT, Moller JT: Postoperative cognitive dysfunction in middle-aged patients. *ANESTHESIOLOGY* 2002; 96:1351–7
2. Moller JT, Cluitmans P, Rasmussen LS, Houx P, Rasmussen H, Canet J, Rabbitt P, Jolles J, Larsen K, Hanning CD, Langeron O, Johnson T, Lauven PM, Kristensen PA, Biedler A, van Beem H, Frigidakis O, Silverstein JH, Beneken JE, Gravenstein JS: Long-term postoperative cognitive dysfunction in the elderly ISPOCD1 study. ISPOCD Investigators. *International Study of Post-Operative Cognitive Dysfunction*. *Lancet* 1998; 351:857–61
3. Culley DJ, Baxter M, Yukhananov R, Crosby G: The memory effects of general anesthesia persist for weeks in young and aged rats. *Anesth Analg* 2003; 96:1004–9
4. Olton DS, Walker JA, Wolf WA: A disconnection analysis of hippocampal function. *Brain Res* 1982; 233:241–53
5. Otto T, Wolf D, Walsh TJ: Combined lesions of perirhinal and entorhinal cortex impair rats' performance in two versions of the spatially guided radial-arm maze. *Neurobiol Learn Mem* 1997; 68:21–31
6. Porter MC, Mair RG: The effects of frontal cortical lesions on remembering depend on the procedural demands of tasks performed in the radial arm maze. *Behav Brain Res* 1997; 87:115–25
7. Borde N, Jaffard R, Beracochea D: Effects of chronic alcohol consumption

or diazepam administration on item recognition and temporal ordering in a spatial working memory task in mice. *Eur J Neurosci* 1998; 10:2380-7

8. Ward MT, Stoelzel CR, Markus EJ: Hippocampal dysfunction during aging II: deficits on the radial-arm maze. *Neurobiol Aging* 1999; 20:373-80

9. Bailey JM: Context-sensitive half-times and other decrement times of inhaled anesthetics. *Anesth Analg* 1997; 85:681-6

10. Rice SA, Fish KJ: Anesthetic metabolism and renal function in obese and nonobese Fischer 344 rats following enflurane or isoflurane anesthesia. *ANESTHESIOLOGY* 1986; 65:28-34

11. Mercier SF, Canini BA, Cespluglio R, Martin S, Bourdon L: Behavioural changes after an acute stress: stressor and test types influences. *Behav Brain Res* 2003; 139:167-75

12. Olton DS, Samuelson RJ: Remembrance of places passed: Spatial memory in rats. *J Exp Psychol Anim Behav Process* 1976; 2:97-116

13. Bailey CH, Bartsch D, Kandel ER: Toward a molecular definition of long-term memory storage. *Proc Natl Acad Sci USA* 1996; 93:13445-52

14. Langer G, Karazman R, Neumark J, Saletu B, Schonbeck G, Grunberger J, Dittrich R, Petricek W, Hoffmann P, Linzmayer L: Isoflurane narcotherapy in depressive patients refractory to conventional antidepressant drug treatment: A double-blind comparison with electroconvulsive treatment. *Neuropsychobiology* 1995; 31:182-94

15. Levin ED, Uemura E, Bowman RE: Neurobehavioral toxicology of halothane in rats. *Neurotoxicol Teratol* 1991; 13:461-70

16. Olney JW, Wozniak DF, Jevtovic-Todorovic V, Farber NB, Bittigau P, Ikonomidou C: Drug-induced apoptotic neurodegeneration in the developing brain. *Brain Pathol* 2002; 12:488-98

17. Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND, Dikranian K, Zorumski CF, Olney JW, Wozniak DF: Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci* 2003; 23:876-82

18. Jevtovic-Todorovic V, Benshoff N, Olney JW: Ketamine potentiates cerebrocortical damage induced by the common anesthetic agent nitrous oxide in adult rats. *Br J Pharmacol* 2000; 130:1692-8

19. Jevtovic-Todorovic V, Wozniak DF, Benshoff ND, Olney JW: A comparative evaluation of the neurotoxic properties of ketamine and nitrous oxide. *Brain Res* 2001; 895:264-7

20. Beals JK, Carter LB, Jevtovic-Todorovic V: Aged rat brain is more sensitive than young rat brain to the neurotoxic effects of combined nitrous oxide and ketamine anesthesia (abstract 889.12). Presented at the Annual Meeting of the Society for Neuroscience, Orlando, Florida, November 2-7, 2002

21. Loguinov AV, Anderson LM, Crosby GJ, Yukhananov RY: Gene expression following acute morphine administration. *Physiol Genomics* 2001; 6:169-81

22. Hanning CD, Blokland A, Johnson M, Perry EK: Effects of repeated anesthesia on central cholinergic function in the rat cerebral cortex. *Eur J Anaesthesiol* 2003; 20:93-7

23. Sarraf-Yazdi S, Sheng H, Miura Y, McFarlane C, Dexter F, Pearlstein R, Warner DS: Relative neuroprotective effects of dizocilpine and isoflurane during focal cerebral ischemia in the rat. *Anesth Analg* 1998; 87:72-8

24. Caramanos Z, Shapiro ML: Spatial memory and *N*-methyl-D-aspartate receptor antagonists APV and MK-801: Memory impairments depend on familiarity with the environment, drug dose, and training duration. *Behav Neurosci* 1994; 108:30-43

25. Abel T, Lattal KM: Molecular mechanisms of memory acquisition, consolidation and retrieval. *Curr Opin Neurobiol* 2001; 11:180-7

26. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anesthesia. *Nature* 1994; 367:607-14

27. McGaugh JL: Memory: A century of consolidation. *Science* 2000; 287:248-51

28. McGaugh JL, Izquierdo I: The contribution of pharmacology to research on the mechanisms of memory formation. *Trends Pharmacol Sci* 2000; 21:208-10

29. Engert F, Bonhoeffer T: Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* 1999; 399:66-70

30. Ramirez-Amaya V, Balderas I, Sandoval J, Escobar ML, Bermudez-Rattoni F: Spatial long-term memory is related to mossy fiber synaptogenesis. *J Neurosci* 2001; 21:7340-8