Anesthesiology 2010; 113:1061–71

Short-term Memory Impairment after Isoflurane in Mice Is Prevented by the α5 γ-Aminobutyric Acid Type A Receptor Inverse Agonist L-655,708

Bechara J. Saab, Ph.D.,* Ashley J. B. MacLean, B.Sc.,† Marijana Kanisek, M.Sc.,‡ Agnieszka A. Zurek, B.Sc.,§ Loren J. Martin, Ph.D.,‖ John C. Roder, Ph.D.,# Beverley A. Orser, Ph.D., M.D.**

ABSTRACT

Background: Memory blockade is an essential component of the anesthetic state. However, postanesthesia memory deficits represent an undesirable and poorly understood adverse effect. Inhibitory α5 subunit–containing γ-aminobutyric acid subtype A receptors (α5GABA$_A$) are known to play a critical role in memory processes and are highly sensitive to positive modulation by anesthetics. We postulated that inhibiting the activity of α5GABA$_A$ receptors during isoflurane anesthesia would prevent memory deficits in the early postanesthesia period.

Methods: Mice were pretreated with L-655,708, an α5GABA$_A$ receptor–selective inverse agonist, or vehicle. They were then exposed to isoflurane for 1 h (1.3%, or 1 minimum alveolar concentration, or air-oxygen control). Then, either 1 or 24 h later, mice were conditioned in fear-associated contextual and cued learning paradigms. In addition, the effect of L-655,708 on the immobilizing dose of isoflurane was studied. Motor coordination, sedation, anxiety, and the concentration of isoflurane in the brain at 5 min, 1 h, and 24 h after isoflurane were also examined.

Results: Motor and sensory function recovered within minutes after termination of isoflurane administration. In contrast, a robust deficit in contextual fear memory persisted for at least 24 h. The α5GABA$_A$ receptor inverse agonist, L-655,708, completely prevented memory deficits without changing the immobilizing dose of isoflurane. Trace concentrations of isoflurane were measured in the brain 24 h after treatment.

Conclusions: Memory deficits occurred long after the sedative, analgesic, and anxiolytic effects of isoflurane subsided. L-655,708 prevented memory deficit, suggesting that an isoflurane interaction at α5GABA$_A$ receptors contributes to memory impairment during the early postanesthesia period.

What We Already Know about This Topic

- Inhaled anesthetics cause persistent postanesthesia learning and memory deficits by unknown mechanisms
- Specific γ-aminobutyric acid (GABA) receptor subtypes involved in memory are modulated by isoflurane, and could be involved

What This Article Tells Us That Is New

- Pretreatment of mice with a selective α5-GABA$_A$ receptor inverse agonist prevented isoflurane-induced memory deficits
- The α5-GABA$_A$ receptor may play a role in postanesthesia memory impairment and its prevention

IT has been widely assumed that the neurodepressive effects of general anesthetics dissipate rapidly and that cognitive faculties promptly return to baseline once the anesthetic has been eliminated. However, observational studies of patients who have undergone cardiac and noncardiac procedures have shown that cognitive decline is present in 31–

◇ This article is featured in "This Month in ANESTHESIOLOGY." Please see this issue of ANESTHESIOLOGY, page 9A.

* Graduate Student, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada, Department of Molecular Genetics, University of Toronto, Toronto, Canada. † Undergraduate Student, Department of Pharmacology and Toxicology, University of Toronto. ‡ Technician, Samuel Lunenfeld Research Institute, Mount Sinai Hospital. § Graduate Student, Department of Physiology, University of Toronto. ‖ Graduate Student, Institute of Medical Science, University of Toronto. # Senior Investigator, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Professor, Departments of Molecular Genetics and Physiology, University of Toronto. ** Professor, Departments of Anesthesia and Physiology, University of Toronto, Department of Anesthesia, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada.

Received from the Department of Anesthesia, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada. Submitted for publication May 16, 2010. Accepted for publication July 28, 2010. Supported by Grant nos. MOP-13239 (to Dr. Roder), MOP-38028, and MOP-79428 (to Dr. Orser) from the Canadian Institute of Health Research (Ottawa, Ontario). Supported also by a Canada Research Chair in Anesthesia (to Dr. Orser) as well as a Canada Research Chair in Learning and Memory (to Dr. Roder).

Address reprint requests to Dr. Orser: Department of Physiology, Room 3318, Medical Sciences Building, 1 King's College Circle, University of Toronto, Toronto, Ontario, M5S 1A8 Canada. beverley.orser@utoronto.ca.

47% of patients at the time of hospital discharge and in 10% of patients at 3 months.[1–3]

The highest incidence of memory impairment occurs in the early postanesthesia period. For example, 47% of elderly patients who underwent general anesthesia for minor surgical procedures exhibit memory deficits for at least 24 h.[4] Declarative or explicit memory (which refers to memory for facts, objects, places, and events) is particularly vulnerable.[1,2,5] The underlying mechanisms, severity, and time course for recovery of memory deficits in the early postanesthesia period remain poorly understood.

In clinical studies, it is not possible to disentangle the effects of anesthetics from other factors that impair memory, such as inflammation, analgesic drugs, and concurrent disease.[6,7] Consequently, animal models are required to identify susceptible cognitive domains and the mechanisms underlying memory deficits after exposure to general anesthetics.

The hippocampus is required for several forms of explicit memory. The temporal stages of explicit memory strongly parallel the stages of synaptic plasticity in the hippocampus.[8] In addition, the acute memory-blocking effects of anesthetics parallel the inhibition of synaptic plasticity in the hippocampus.[9]

The current study used a well-characterized behavioral model of hippocampus-dependent, fear-associated learning to study the mechanisms underlying short- and long-term memory deficits after exposure to inhaled anesthesia. Within the current context of the study, *short-term memory* refers to memory that lasts for minutes whereas *long-term memory* lasts for days.

Early and late forms of memory were studied because they are known to depend on different neurotransmitter receptors, intracellular signaling pathways, and regulators of gene expression.[8,10–12] Specifically, short-term memory involves changes in the strength of preexisting synaptic connections and modulation of existing proteins. Long-term memory requires gene transcription, the production of new proteins, restructuring of synapses, and growth of new synaptic connections.[8]

The goal of the current study was to determine whether brief exposure to a general anesthetic produces deficits in short- and long-term memory. We also sought to develop a pharmacological strategy to prevent memory deficits based on anesthetic interactions at a receptor that plays a central role in memory pathways.

Most anesthetics cause deficits in memory and synaptic plasticity, at least in part, by increasing the activity of inhibitory γ-aminobutyric acid subtype A ($GABA_A$) receptors.[13] $GABA_A$ receptors are heteromeric complexes composed of multiple subunits (α1–6, β1–3, γ1–3, δ, ε, θ, π, ρ1–3). In particular, the activity of $GABA_A$ receptors, which contain the α5 subunit (α5$GABA_A$) receptors, regulate synaptic plasticity and hippocampus-dependent memory.[6,7,14,15] α5$GABA_A$ receptors set the threshold for the induction of plasticity in pyramidal neurons by attenuating excitatory input.[9] Memory blockade during anesthesia has been attributed, in part, to increased α5$GABA_A$ receptor activity.[9,16,17]

In the current study, we first sought to characterize memory deficits in the postanesthesia period and determine whether such deficits were dissociated from impairment of motor function, anxiolysis, and nociception. Next, we tested the hypothesis that inhibiting α5$GABA_A$-receptor activity during anesthesia prevents memory deficits in the early postanesthesia period. Specifically, we tested whether L-655,708, an inverse agonist with high selectivity for α5$GABA_A$ receptors, prevents memory deficits that occur after isoflurane anesthesia.[18] Short- (30 min after conditioning) and long-term (2 days after conditioning) memory were measured after 1 h of isoflurane exposure. Results showed that mice rapidly recovered motor coordination, locomotion, and nociception after exposure to isoflurane; however, a deficit in contextual fear memory that persisted for up to 24 h could be prevented by pretreatment with L-655,708.

Materials and Methods

Animals

Animal care and experimental protocols were approved by the University of Toronto Animal Care Committee (Toronto, Ontario,Canada) and conformed to guidelines set by the Canadian Council on Animal Care. Male and female adult (aged 8–16 weeks) C57BL6/J mice (Charles River Laboratories, Saint-Constant, Quebec, Canada) were studied. The mice were housed five to a cage with free access to food and water. The temperature (22°C) and reverse light-dark cycle (lights on at 7:00 AM; lights off at 7:00 PM) of the room were controlled. To reduce variability in learning and memory performance, and to prevent acute stress reactions during conditioning, all mice were handled daily for 10 min for a minimum of 1 week before behavioral experiments were started.[19]

The experimenters, who scored behavioral performance, were blinded to group assignments. To avoid subjecting the mice to multiple tests, different groups of mice were used to study motor coordination, nociception, core temperature during anesthesia, arterial blood gases, and the concentration of isoflurane in the brain. Anxiety levels were studied using the elevated plus maze (EPM) in same group of mice used to study fear conditioning.

Isoflurane Anesthesia

Mice selected at random received either L-655,708 (0.7 mg/kg in 10% dimethyl sulfoxide 2 μl/g) or vehicle (10% dimethyl sulfoxide) by subcutaneous injection 10 min before exposure to isoflurane or vehicle gas. During isoflurane exposure, individual mice were placed in an airtight clear acrylic chamber (27 × 10 × 10 cm). The chamber was preflushed with a vehicle-gas mixture (30% oxygen [O_2] in air delivered at 1 l/min) that did, or did not, contain isoflurane. The desired concentration of isoflurane was set on the vaporizer as 1 minimum alveolar concentration (MAC) or 1.33% for C57BL6/J mice.[20] Isoflurane concentrations, oxygen, and carbon dioxide in the chamber were continuously

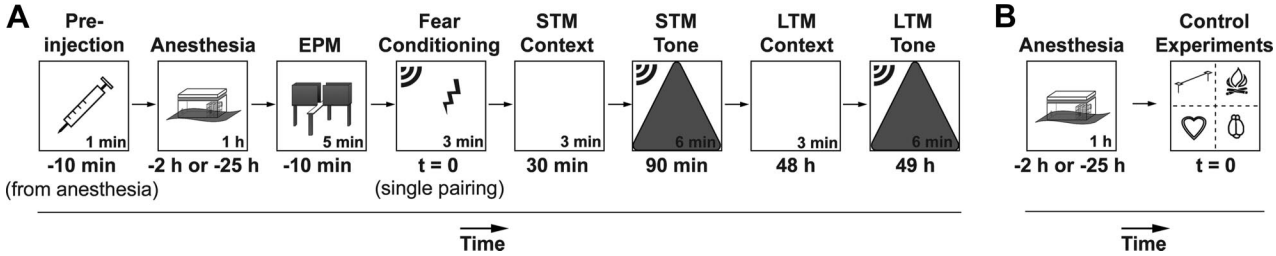

Fig. 1. Experimental paradigm timeline. (*A*) Each stage of the experimental paradigm is represented chronologically by boxes and cartoons. Relative time is indicated below the boxes (t = 0, start of fear conditioning training). The duration of each stage is inset at the bottom left. Before fear conditioning, test subjects were injected with L-655,708 or vehicle and 10 min later anesthetized with 1.0 minimum alveolar concentration (MAC) isoflurane in 30% oxygen (O_2). Four groups of isoflurane-treated subjects were used (1-h recovery + L-655,708; 1-h recovery + vehicle; 24-h recovery + L-655,708; 24-h recovery + vehicle). Two O_2 control groups were used (1-h recovery + L-655,708; 1-h recovery + vehicle). 10 min before fear conditioning, subjects were examined in the elevated plus maze (EPM). Subjects were tested for short-term memory (STM) and long-term memory (LTM) of the context and tone. (*B*) Control experiments: balance beam, tail flick (fire), blood gas (heart), and isoflurane brain quantification (brain, dorsal view) experiments were performed independently from learning and memory experiments.

analyzed with a commercial gas analyzer (Datex-Ohmeda [Canada], Inc., Mississauga, Ontario, Canada). To prevent hypothermia during anesthesia, the floor of the chamber was warmed with a heating blanket. During the recovery phase (after isoflurane or vehicle gas treatment), the mouse was taken from the gas chamber and placed in a second heated clear acrylic chamber for 45 min. The mouse was then either returned to its home cage and allowed to recover for 24 h (24-h groups) or taken to a holding cage in an adjacent room (1-h groups). For continuity, and to ensure that the experimenter who performed the fear-conditioning studies was blinded to group assignment, mice that were allowed to recover for 24 h were placed back in a heated chamber for 45 min before the behavioral experiments. Figure 1A and 1B summarize the time course of the experimental procedures.

The sample size selected for fear-associated memory studies was determined by an independent cohort of 14 mice (7 male, 7 female) that demonstrated a deficit in short-term contextual fear conditioning 24 h after exposure to isoflurane compared with oxygen-treated controls. In control animals, the mean freezing score was $\mu_o = 57.8\%$ whereas the SD was $\sigma_o = 23.0\%$. In mice treated with isoflurane and studied 24 h after the anesthetic was terminated, the freezing score was $\mu_1 = 32.8\%$ and the SD was $\sigma_1 = 20.8\%$. A sample size calculation, based on an α value of 0.05, a 1-β value equal to 80%, and using a one-tailed test with an n of 10. The sample size was calculated using a formula where $n = (z_{1-\alpha} + z_{1-\beta})^2(\sigma_o^2 + \sigma_1^2)/(\mu_o - \mu_1)^2$.

L-655,708 Effects on MAC

Mice selected at random received either L-655,708 (0.7 mg/kg) or vehicle (10% dimethyl sulfoxide) by subcutaneous injection 10 min before exposure to isoflurane. The affinity of L-655,708 for α5GABA$_A$ receptors is 50- to 100-fold greater than its affinity for α1, α2, and α3 subunit receptors.[18] All available inverse agonists for α5GABA$_A$ receptors bind to other benzodiazepine-sensitive GABA$_A$ receptor subtypes, albeit at lower affinity, and modify receptor function at higher concentrations. The subtype selectivity of L-655,708 is attributed to a higher affinity for α5GABA$_A$ receptors because the efficacy of this compound is similar for other receptor subtypes to which it binds.[18] Consequently, to ascribe an effect of L-655,708 to α5GABA$_A$ receptors, a careful selection of the concentration is required. The concentrations of L-655,708 used in the current study were selected on the basis of *in vivo* binding data, pharmacokinetic analyses, and previous memory studies. We estimated that, at 30 min after injection, L-655,708 at 0.7 mg/kg, administered intraperitoneally, would result in 60–70% occupancy of α5GABA$_A$ receptors *in vivo* with limited binding to α1, α2, and α3 subunit–containing GABA$_A$ receptors and no significant off-target behavioral effects, such as sedation and motor impairment.[21]

The tail-clamp withdrawal assay was used to determine whether L-655,708 influenced the potency of isoflurane for prevention of a motor response to noxious stimulus.[22] After equilibration with 1.1% isoflurane, a hemostat was applied to the tail and the mouse was assessed for purposeful movement in response to the tail-clamp. The dose of isoflurane was adjusted by approximately 0.15%, up- or downward, depending on the response, and equilibrated for 15 min. This method was continued until the isoflurane concentrations that prevented and produced movement were determined. MAC value was calculated as the mean value of these two concentrations. Mice were treated in a heated chamber and the inspired concentration of isoflurane was continuously analyzed with a commercial gas analyzer (Datex-Ohmeda [Canada], Inc.). Data were examined using procedures described in Statistical Analysis.

Core Temperature during Anesthesia

Rectal temperature was studied in a separate group of mice to ensure that it did not drop substantially during isoflurane anesthesia. Mice were placed in an airtight clear acrylic chamber for administration of the anesthetic. Rectal probes were inserted after the mice lost their righting reflex. Rectal temperature was inserted to measure temperature between 5 min and 1 h. The rectal probe was not inserted before the 5-min mark to ensure that mice were adequately anesthetized and not discomforted.

Analysis of Blood Gases

Mice were anesthetized in a clear acrylic chamber that was flushed with isoflurane (1 MAC in 30% O_2) for either 5 min or 1 h, then placed in the supine position on a stereotactic frame. Isoflurane (1.0 MAC) was administered *via* a nose cone. A needle was carefully inserted into the heart and blood was extracted into a heparinized syringe. The collected blood was immediately placed on ice and transported to a blood gas analyzer (ABL 700 series; Radiometer, Copenhagen, Denmark).

Concentration of End-tidal Isoflurane during Recovery

Immediately after isoflurane anesthesia, several mice were transferred to a heated recovery chamber and a sampling catheter was positioned as close as possible to the snout of each mouse. The concentration of isoflurane in the expired gas was measured until it could no longer be detected (approximately 7 min).

Isoflurane Concentration in Brain Tissue

After a designated recovery time, mice were sacrificed by cervical dislocation. The brains were rapidly removed at room temperature and placed in a gas-tight syringe that contained microbeads coated with tetrafluoroethylene. The whole brain was immediately crushed with two strokes of the plunger and the syringe was then sealed. Isoflurane was measured using gas chromatography, as described previously.[23–25] This method uses headspace gas chromatography based on previous measurements of the gas-brain coefficient. The methods are similar to that described for measurement of inhaled anesthetics in blood.[26]

Fear-conditioning Studies

Testing of memory function was performed using two fear-conditioning paradigms. These tests study the ability of mice to learn and remember an association between an aversive stimulus (foot shock) and nonaversive, or neutral, stimuli (environmental context *vs*. auditory cue). Different groups of mice were used to study associated fear conditioning at 1 h and 24 h after the end of isoflurane or vehicle treatment. Each mouse was introduced to the fear-conditioning chamber (Process Control Fear Conditioning Monitor 303410; Technical & Scientific Equipment Systems, Inc., Chesterfield, MO). The chamber (50 × 15 × 15 cm) was illuminated with an interior overhead light (50 lx) and was equipped with a stainless steel grid floor connected to a constant-current shock generator. Each mouse was allowed to explore the chamber for 3 min before presentation of a 2-Hz pulsating tone (80 dB, 3,600 Hz) that persisted for 30 s. The tone was followed immediately by a mild foot shock (0.7 mA for 0.5 s). The mouse was allowed to explore the chamber another 30 s after the shock to study postshock freezing. Assessment of learning and memory was performed by measuring the amount of time the mouse demonstrated "freezing behavior," defined as a completely immobile posture, except for respiratory efforts. Freezing was scored using Observer software (Noldus Information Technology, Wageningen, The Netherlands), by an experimenter who was blinded to group assignments. Before each new training session, the chamber was cleaned with 70% ethanol to mask and eliminate odors from the previous mouse. At the end of the experiment, the mouse was removed from the chamber and housed singly in a temporary cage located in a separate room until completion of the short-term memory testing, after which it was returned to its home cage.

Short-term memory for contextual learning was probed 30 min after training by reexposing mice to the same fear-conditioning chamber for 3 min whereas long-term memory was studied 2 days later *via* conditioning chamber reexposure. Short-term memory for cued learning was determined 90 min after training by allowing the mice to explore a modified chamber scented with 5% acetic acid. The chamber contained a plastic floor and cardboard walls. After 3 min of chamber exploration, during which nonspecific freezing was measured, the same 2-Hz pulsating tone (80 dB, 3,600 Hz) was presented for 3 min. To study recall, freezing scores were measured during tone presentation. Long-term memory for cued learning was determined by introducing the mice into the modified chamber and presenting the tone 2 days after conditioning.

Elevated Plus Maze

Anxiety levels were studied using an EPM consisting of a clear acrylic apparatus in the shape of a cross (45 × 7 cm with a central region of 7 × 7 cm). Two opposing arms were enclosed with opaque white plastic walls (28 cm high, closed arms), whereas the other two opposing arms were left open (open arms). Ten minutes before the mice were placed in the fear-conditioning chamber, they were observed for 5 min in the EPM. An observer blinded to group assignment recorded the position of each mouse on the maze using Observer software. The time spent in the center and in the open and closed arms were measured during 5 min. In addition, motor activity was quantified to study locomotion. A 5-min interval was granted between the EPM test and fear-conditioning training.

Tail Flick

Mice were held gently by the scruff and allowed free movement of the tail. The tip of the tail was inserted to approximately 1 cm depth into a beaker of water (49°C). Latency for the mouse to remove its tail from the water was recorded and used as an indication of nociception.

Balance Beam

To determine whether motor coordination was impaired 1 h after exposure to isoflurane, mice were selected at random and a double crossover study was undertaken using a separate group of mice. Mice were pretreated with isoflurane or vehicle for 1 h and then given 1 h to recover before experimentation on a balance beam. One hour after exposure to isoflurane or vehicle, the mice were placed on a wooden platform (15 × 15 cm) elevated 40 cm from the ground and attached to an identical elevated platform by a wooden beam 2 cm in diameter and 1.2 m long. Indicators of motor coordination

and mobility included the time necessary for mice to cross the beam spontaneously, and the number of times the hindfoot slipped off the beam. Average values of three repetitions were reported for each mouse. The mice were pretrained on the apparatus 1 day before the experiment.

Statistical Analysis

All statistics were carried out using Statistica (StatSoft, Inc., Tulsa, OK) software on a standard personal computer. Groups examined in the EPM and fear conditioning training were compared using a two-way ANOVA followed by *post hoc* Least Significant Difference (LSD) tests, using exposure to isoflurane and pretreatment with L-655,708 as the two factors. The remaining experiments were compared using one-way ANOVA followed by *post hoc* LSD tests, where the single factor was exposure to isoflurane, pretreatment with L-655,708, or recovery time after isoflurane anesthesia. Differences between groups were considered statistically significant at $P < 0.05$. All analyses were performed using two-tailed tests. When testing for effects of isoflurane, comparisons were made against the oxygen-treated group. Differences are represented in the figures using asterisks. When testing for effects of L-655,708, comparisons were made against the corresponding vehicle-treated groups. These differences are represented in the figures using daggers.

The dose-response plot of MAC was fit by nonlinear regression analysis using SigmaPlot (Systat Software, Inc., San Jose, CA) to estimate the concentration that caused 50% of the maximum effect (EC_{50}): $Y = \text{Control} + (I_{max} - \text{Control})/(1 + 10^{((\text{Log } EC_{50} - X) \cdot \text{Hill slope})})$, where Y is the response, I_{max} is the maximum response, and X is the logarithm of the concentration.

Results

Mice were pretreated with L-655,708 or vehicle then exposed to isoflurane (1 MAC or vehicle) for 1 h. After a 1- or 24-h recovery period, mice were trained to associate a foot shock with the context of a chamber (contextual fear learning) and an audible tone that was presented immediately before the shock (cued-fear conditioning).

Short- and Long-term Memory after Isoflurane

Short-term memory for contextual learning was studied by reintroducing the mice to the training chamber 30 min after conditioning. Those trained 1 h after isoflurane exhibited a memory deficit evidenced by lower mean ± SEM freezing scores compared with oxygen-treated controls (54.6 ± 5.9%, n = 11 *vs.* 82.8 ± 4.31%, n = 12; $P = 0.003$; fig. 2A). Mice trained 24 h after isoflurane also exhibited lower freezing scores compared with controls (51.6 ± 8.0%, n = 12; $P = 0.0008$; fig. 2A). Thus, a short-term contextual memory deficit after isoflurane persists for at least 24 h.

The use of L-655,708 completely reversed short-term memory deficit for contextual learning. Freezing scores at 1 and 24 h after isoflurane (75.5 ± 8.6%, n = 9; $P = 0.04$; 81.9 ± 5.6%, n = 12; $P = 0.001$, respectively; fig. 2A) were similar to oxygen-treated control mice. It is important to note that L-655,708 had no effect on freezing in the oxygen-treated mice, suggesting that a generalized enhancement of memory does not account for prevention of the postisoflurane memory deficits (fig. 2A). *Post hoc* analysis revealed a main effect of isoflurane ($F_{2,61} = 3.9$, $P = 0.03$), L-655,708 ($F_{1,61} = 9.2$, $P = 0.004$), and their interaction ($F_{2,61} = 3.6$, $P = 0.03$), whereas L-655,708 had no effect on oxygen-treated mice (80.1 ± 6.4%, n = 11; $P = 0.76$; fig. 2A).

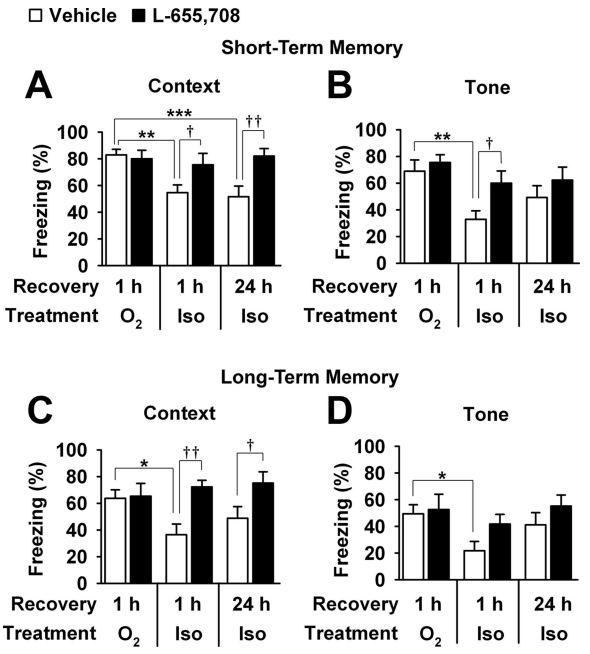

Fig. 2. Isoflurane causes impairment in short- and long-term memory that is prevented by preadministration of L-655,708. Differences are represented using asterisks. When testing for effects of L-655,708, comparisons were made against the corresponding vehicle-treated groups; differences are represented using daggers. (*A*) Freezing behavior to the context studied 30 min after training. (*B*) Freezing behavior to the audible cue 90 min after training. (*C*) Freezing behavior to the context 2 days after training. (*D*) Freezing behavior to the audible cue 2 days after training. (1-h oxygen [O_2], vehicle: n = 12; 1-h O_2, L-655,708: n = 11; 1-h isoflurane (Iso), vehicle: n = 11; 1-h isoflurane, L-655,708: n = 9; 24-h isoflurane, vehicle: n = 12; 24-h isoflurane, L-655,708: n = 12.) * $P < 0.05$ and ** $P < 0.01$, *** $P < 0.001$ compared with O_2-treated subjects, 1 h, vehicle; † $P < 0.05$ and †† $P < 0.01$ compared with vehicle control.

Short-term memory of the auditory tone was studied by placing mice in a novel context and reintroducing the audible tone 90 min after conditioning. Mice trained 1 h after isoflurane had lower freezing scores relative to oxygen-treated controls (35.7 ± 6.43%, n = 11 *vs.* 68.9 ± 8.3%, n = 12; $P = 0.002$; fig. 2B). These results are consistent with a deficit in short-term memory for cued learning. Mice trained 24 h after isoflurane exhibited no significant memory deficit to auditory tone (49.1 + 8.8%, n = 12, $P = 0.08$; fig. 2B). L-655,708 prevented short-term memory deficit to the tone in mice trained 1 h

after isoflurane (49.1 + 8.8%, n = 12; $P = 0.03$, fig. 2B), but it had no effect on auditory recall in mice conditioned 24 h after exposure to isoflurane. Analysis of cued memory at 90 min revealed a main effect of isoflurane ($F_{2,61} = 4.9$, $P = 0.01$) and L-655,708 ($F_{1,61} = 5.4$, $P = 0.02$), but not for the interaction ($F_{2,61} = 0.8$, $P = 0.5$).

Long-term memory for contextual learning after isoflurane was studied by reintroducing the mice to the fear-conditioning chamber 2 days after training. Mice trained 1 h after isoflurane showed lower freezing scores than oxygen-treated controls (36.4 ± 26.9%, n = 11; *vs.* 63. 7 ± 6.5%, n = 12; $P = 0.02$; fig. 2C). This memory deficit was completely reversed by pretreatment with L-655,708 (72.3 ± 4.9%, n = 9; $P = 0.004$, fig. 2C). Among mice trained 24 h after exposure to isoflurane, memory was significantly improved by L-655,708 compared with vehicle-treated controls (75.2 ± 8.3%, n = 12; $P = 0.02$). However, mice trained 24 h after isoflurane revealed only a trend toward lower freezing scores compared with air-oxygen controls (48.9 ± 8.6%, n = 12; $P = 0.18$). Analysis revealed a main effect of L-655,708 ($F_{1,61} = 9.6$, $P = 0.003$), but not of isoflurane ($F_{2,61} = 1.0$, $P = 0.4$) or the interaction ($F_{2,61} = 2.1$, $P = 0.1$).

Long-term memory for the auditory tone was studied 2 days after fear conditioning. Long-term memory was reduced in mice trained 1 h after isoflurane exposure (21.8 ± 23.1%, n = 11 *vs.* 49.5 ± 6.8%, n = 12; $P = 0.02$). Mice trained 24 h after isoflurane showed no significant reduction in freezing scores (41.2 ± 31.2%, n = 12; $P = 0.48$; fig. 2D). L-655,708 did not reverse the long-term deficit in cued fear conditioning in the mice trained 1 h after isoflurane exposure (41.7 ± 7.3%, n = 9; $P = 0.13$).

L-655,708 Effects on MAC and Hypnosis

It is possible that L-655,708 modified other components of the anesthetic state during isoflurane anesthesia, including immobility and sedation. However, the tail-clamp assay showed that L-655,708 did not change the MAC value for isoflurane (fig. 3). The immobilizing dose of isoflurane was similar in mice treated with L-655,708 (1.33 ± 0.02, n = 17) or vehicle (1.33 ± 0.02, n = 17, $P > 0.05$). In addition, there was no significant difference in the time to self-right after the immobilizing dose of isoflurane was administered (vehicle: 153 ± 26 s, L-655,708: 151 ± 27 s; n = 9 for both groups, $P > 0.05$). Thus, L-655,708 did not modify the "depth of anesthesia" as indicated by the immobilizing dose of isoflurane or the latency to recovery of the righting reflex after isoflurane exposure.

L-655,708 Does Not Alter Baseline Freezing Scores

The reduction in freezing scores observed 24 h after isoflurane was not associated with residual sedation or deficits in locomotion, as evidenced by the lack of change in baseline freezing scores. Baseline freezing scores, measured during the training phase immediately before tone presentation, were identical to those of controls studied 1 h after oxygen

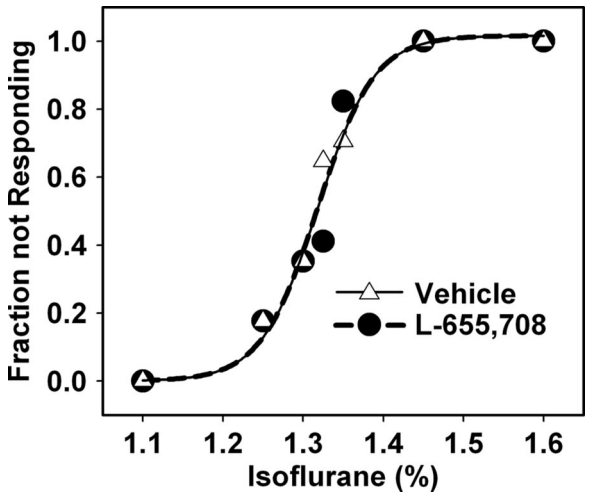

Fig. 3. L-655,708 does not affect minimum alveolar concentration (MAC) in mice. Preinjection of L-655,708 did not alter the MAC value of isoflurane as measured using the tail-clamp assay compared with vehicle-treated controls. Dose-response plots for the immobilizing dose of isoflurane revealed no difference in the EC_{50} values estimated from the fitted curves (mean ± SEM, 1.32 ± 0.048 *vs.* 1.32 ± 0.048, n = 17 per group) for L-655,708-treated and vehicle-control mice, respectively.

alone (n = 12; fig. 4A, Context), 1 h after isoflurane (n = 11, $P = 1.0$), and 24 h after isoflurane (n = 12; $P = 1.0$). No main effects were detected (isoflurane: $F_{2,61} = 0.8$, $P = 0.4$; L-655,708: $F_{1,61} = 3.2$, $P = 0.08$; isoflurane × L-655,708: $F_{2,61} = 0.8$, $P = 0.4$). It is also notable that L-655,708 significantly increased freezing to context during training in oxygen-treated controls (n = 11; $P = 1.0$), 1 h after isoflu-

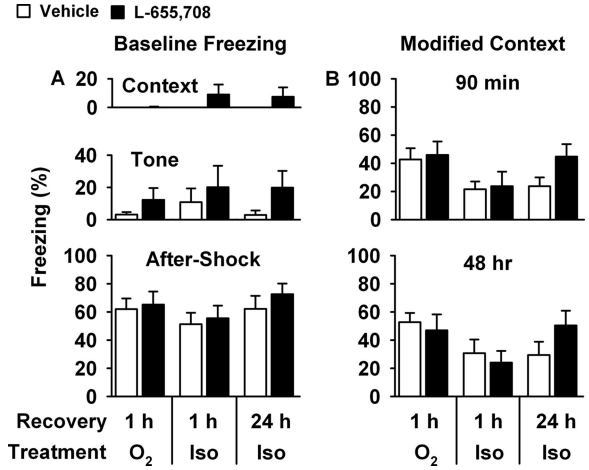

Fig. 4. Isoflurane and L-655,708 do not affect baseline freezing behavior, motor function, or nociception in mice. (*A*) Baseline freezing before tone-shock pairing in the fear-conditioning chamber before (Context) and during (Tone) tone presentation as well as immediately after delivery of mild foot shock (After-shock). (*B*) Freezing in a modified context at the indicated time intervals after training. (1-h oxygen [O_2], vehicle: n = 12; 1-h O_2, L-655,708: n = 11; 1-h isoflurane (Iso), vehicle: n = 11; 1-h isoflurane, L-655,708: n = 9; 24-h isoflurane, vehicle: n = 12; 24-h isoflurane, L-655,708: n = 12).

rane (n = 9; $P = 0.11$), and 24 h after isoflurane (n = 12; $P = 0.15$). Likewise, baseline freezing scores to the tone, measured during the training phase immediately before shock delivery, were not significantly different in oxygen-treated controls (n = 12; fig. 4A, Tone), 1-h isoflurane group (n = 11; $P = 0.4$), or the 24-h isoflurane group (n = 12; $P = 1.0$). In addition, L-655,708 alone did not alter baseline motor activity; no change in freezing to the yet-unpaired tone was shown in oxygen-treated controls (n = 11; $P = 0.4$), at 1 h after isoflurane (n = 9; $P = 0.4$), or at 24 h after isoflurane (n = 12; $P = 0.12$). In addition, freezing scores measured immediately after foot shock presentation did not differ among oxygen-treated subjects (n = 12; fig. 4A, Aftershock), subjects given 1 h to recover from isoflurane anesthesia (n = 11; $P = 0.79$), or subjects given 24 h to recover after isoflurane anesthesia.

Equally important, L-655,708 did not affect postshock freezing in the oxygen group (n = 12; $P = 0.8$), 1-h isoflurane group (n = 11; $P = 0.74$), or 24-h isoflurane group (n = 12; $P = 0.4$), indicating that the ability of mice to sense noxious stimulus was unaltered. Finally, isoflurane and L-655,708 did not promote nonspecific freezing, as no differences in freezing to the modified context at 90 min were detected (isoflurane: $F_{2,60} = 3.7$, $P = 0.03$; L-655,708: $F_{1,60} = 1.7$, $P = 0.2$; isoflurane × L-655,708: $F_{2,60} = 0.9$, $P = 0.4$) among the three groups injected with vehicle (oxygen-treated, n = 12; 1-h isoflurane, n = 11; $P = 0.06$; 24-h isoflurane, n = 12; $P = 0.09$; fig. 4B) or L-655,708 (oxygen-treated, n = 11; $P = 0.8$; 1-h isoflurane, n = 9; $P = 0.9$; 24-h isoflurane, n = 12; $P = 0.06$). Likewise, no differences in freezing to the modified context after 2 days were detected (isoflurane: $F_{2,61} = 2.6$, $P = 0.08$; L-655,708: $F_{1,61} = 0.2$, $P = 0.7$; isoflurane × L-655,708: $F_{2,61} = 1.4$, $P = 0.3$) among the three groups injected with vehicle (oxygen-treated, n = 12; 1-h isoflurane, n = 11, $P = 0.7$; 24-h isoflurane, n = 12; $P = 0.07$) or L-655,708 (oxygen-treated, n = 11; $P = 0.7$; 1-h isoflurane, n = 9; $P = 0.6$; 24-h isoflurane, n = 12; $P = 0.1$).

Anxiety in Isoflurane-treated Mice

General anesthetics modify a variety of behavioral endpoints that could confound studies of fear memory, including anxiety, motor coordination, and nociception. Therefore, additional control experiments were performed to determine whether these behavioral endpoints were modified 1 and 24 h after anesthesia. To measure anxiety, an EPM trial was performed 10 min before fear-conditioning training. In this task, performance was similar among all groups, indicating that anxiety was not affected. For example, with regard to time spent in closed arms data were as follows: isoflurane: $F_{1,63} = 2.0$, $P = 0.2$; L-655,708: $F_{1,63} = 1.2$, $P = 0.3$; isoflurane × L-655,708: $F_{2,60} = 0.06$, $P = 0.8$ (figs. 5A, 5B, and 5C). In the EPM, the total number of visits to the middle area (the standard measure of activity) was not statistically different for any of the study groups (1-h O_2, vehicle: mean ± SD = 17.2 ± 2.7; 1-h O_2, L-655,708: 19.5 ± 8.5, $P = 0.97$; 1-h isoflurane, vehicle: 19.9 ± 4.6, $P = 1.0$; 1-h isoflurane, L-655,708: 17.3 ± 7.0, $P = 0.99$; 24-h isoflurane, vehicle: 18.1 ± 5.3, $P = 1.0$; 24-h isoflurane, L-655,708: 13.6 ± 7.1, $P = 0.71$), suggesting that the sedative effects of isoflurane did not confounded memory studies. Although we observed a decrease in time spent in the open arm for mice given 24 h to recover from isoflurane and pretreated with L-655,708, there was no associated change in the amount of time spent in the closed arms or a reduction in the total number of crossings. Moreover, because neither isoflurane nor L-655,708 alone had any effect on anxiety, and, in combination, they had no effect when mice we allowed to recover from isoflurane for 1 h before experimentation, differences in anxiety cannot account for the observed isoflurane-induced memory impairment and prevention by L-655,708.

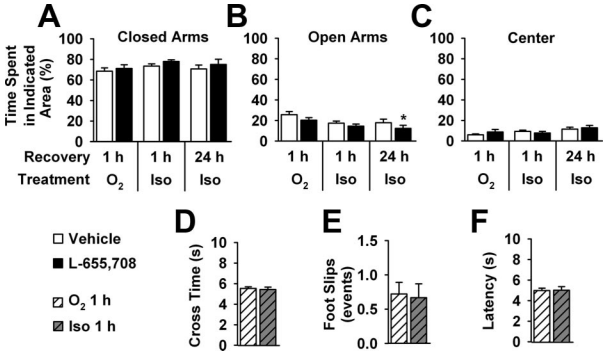

Fig. 5. Other effects of isoflurane (Iso) subside by 1 h after anesthesia. Differences are represented using asterisks. (*A–C*) Amount of time spent in the indicated region of the elevated plus maze (oxygen [O_2], 1 h, vehicle: n = 12; O_2, 1 h, L-655,708: n = 11; isoflurane, 1 h, vehicle: n = 11; isoflurane, 1 h, L-655,708: n = 9; isoflurane, 24 h, saline: n = 12; isoflurane, 24 h, L-655,708: n = 12). (*D*) Time required to cross the elevated beam. (*E*) Number of times hind foot slipped when crossing elevated beam. (*F*) Latency to flick tail away from a hot water bath (n = 6 per group). * $P < 0.05$ compared with O_2, 1 h, saline.

Motor Performance and Nociception in Isoflurane-treated Mice

In a separate group of mice, motor coordination and agility were studied with a balance beam test 1 h after exposure to isoflurane or vehicle. No differences in crossing times (vehicle: n = 12, isoflurane: n = 12; $F_{1,22} = 0.1$, $P = 0.70$; fig. 5D) or the number of foot slips (vehicle: n = 12, isoflurane: n = 12; $F_{1,22} = 0.004$, $P = 0.84$; fig. 5E) were observed between study groups.

Nonassociative fear learning is correlated with the intensity of the electric shock.[27] therefore, it was important to ensure that all groups perceived the same strength of stimulus. Thus, nociception was studied in a separate group of mice using the tail flick assay 1 h after exposure to isoflurane or vehicle. No differences were detected in the latency to tail flick (vehicle: n = 12, isoflurane: n = 12; $F_{1,22} = 0.007$, $P =$

Table 1. Blood Gas Analysis for Mice Anesthetized with Isoflurane for 5 *versus* 60 min

Measure	5 min (n = 6)	60 min (n = 6)	*P* Value
pH	7.28 ± 0.02	7.23 ± 0.08	0.51
pCO_2 (mmHg)	48.6 ± 2.02	54.2 ± 12.3	0.41
pO_2 (mmHg)	142 ± 16	193 ± 31	<0.001
HCO_3 (M)	22.0 ± 0.8	21.9 ± 2.5	0.78

0.94; fig. 5F), a result consistent with the observation that all groups had similar freezing scores after receiving foot shock.

Blood Gas Analysis

Because inhaled anesthetics depress respiration, one could argue hypoxic brain injury contributed to deficits in hippocampus-dependent memory performance in the experimental paradigm. To ensure that hypoxia was not a contributing factor, arterial blood gases were analyzed in separate groups of mice 5 min and 1 h after isoflurane anesthesia (1 MAC in 30% O_2). Hypoxia did not occur (table 1). In addition, similar values for pH, concentration of bicarbonate, and partial pressure of carbon dioxide and oxygen were obtained (table 1).

Isoflurane Concentration in the Brain 1 and 24 h after Anesthesia

The true clearance rate of isoflurane from the mammalian brain remains unknown. At least two rates of clearance have been observed, including one for clearance from the blood and the other for clearance from tissues, particularly individuals with high fat content.[28] Isoflurane concentrations have not been previously measured in mice 24 h after treatment and are commonly assumed to be negligible. Thus, we measured the concentration of isoflurane in the brains of mice 1 and 24 h after isoflurane anesthesia using gas chromatography. In addition, as a positive control, the concentration of isoflurane in the brain was measured 5 min after treatment. The isoflurane concentration in the brain was several times higher at 5 min after treatment than at 1 h after treatment as predicted (5 min: 0.814 ± 0.194%, n = 4; 1 h: 0.034 ± 0.012%, n = 6; $P = 0.0007$; fig. 6A). Surprisingly, at 24 h, residual concentrations of isoflurane were detected in five of six brains (0.0095 ± 0.0006%, n = 6; $P = 0.0005$). Of these five brains, two showed higher concentrations (0.0222, 0.0311%) than the other three (0.0006, 0.0012, and 0.0019%). One brain had zero detectable isoflurane. Likewise, no isoflurane was detected in the brains of mice exposed to vehicle alone (n = 4). The limit of isoflurane detection is approximately 0.0001%. The concentration of isoflurane in expired gas, measured immediately after the mice were removed from the chamber, decreased to an undetectable level within 7 min (fig. 6B). Not surprisingly, the ability to detect isoflurane in the expired gas failed to correlate with the detection of isoflurane in the brain, suggesting the existence of separate rates of clearance.

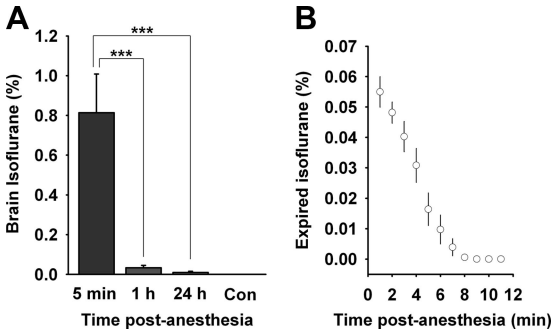

Fig. 6. Isoflurane pharmacokinetics. Differences are represented using asterisks. (*A*) Gas chromatographic quantification of isoflurane from whole mouse brain (5 min: n = 4; 1 h: n = 6; 24 h: n = 6; vehicle control, n = 4). (*B*) Isoflurane clearance from expired gases (n = 6). Con = control, *** $P <$ 0.001

Core Temperature

Rectal temperature was 37.8 ± 0.4°C 5 min after introducing the mice to the anesthetic chamber; 37.8 ± 0.4°C, 1 h after induction (n = 6 per group; $F_{1,10} = 0.02$, $P = 1.0$). The corresponding chamber temperatures were 33.6 ± 0.4°C and 33.4 ± 0.5°C, respectively (n = 6 per group, $F_{1,10} = 0.06$, $P = 0.8$).

Discussion

The current study demonstrates a robust memory deficit for hippocampus-dependent learning that persists for at least 24 h after a relatively brief exposure to isoflurane anesthesia in healthy adult mice. Memory impairment was dissociated from other residual effects of isoflurane, including analgesia, sedation, anxiolysis, and motor impairment. Pretreatment with L-665,708 prevented deficits in short- and long-term memory for contextual learning without altering baseline memory behavior or motor scores. Gas chromatographic analysis revealed undetectable or trace concentrations of isoflurane 24 h after anesthesia.

The detection of residual brain concentrations of isoflurane 24 h after anesthesia was unexpected because this time interval is generally considered sufficient to avoid the confounding effects of residual anesthetic on neurobehavioral performance.[29] Isoflurane undergoes minimal biodegradation (less than 0.2% is metabolized) and nearly 100% of isoflurane can be recovered in expired gas.[30] The presence of trace levels of isoflurane raises the possibility that a direct effect of isoflurane on neuronal networks causes postanesthesia memory deficits. However, the best available evidence from previous studies indicates that much higher concentrations of isoflurane are required to block fear-conditioned memory.[31,32] Isoflurane concentrations as high as 0.6% are required to impair the freezing response when administered during contextual fear learning.[31] In addition, threshold concentrations of four commonly used anesthetics that impaired memory performance in rats during fear conditioning were 0.2% for isoflurane, 0.3% for sevoflurane, 0.3% for

halothane, and 0.44% for desflurane.[32] Isoflurane concentrations measured in the current study at 24 h after anesthesia were orders of magnitude lower than those shown to impair memory directly. Consequently, the isoflurane detected at 24 h most likely represent an incidental finding rather than the direct cause of memory impairment. Instead, postanesthesia memory deficits more likely result from yet-to-be identified processes initiated during exposure to high, "anesthetic" drug doses. An alternative hypothesis is that the initial exposure to a high concentration of isoflurane (1 MAC) preconditioned or primed the neuronal circuitry, rendering it sensitive to trace concentrations of isoflurane in the postanesthesia period. These hypotheses cannot be resolved with the current data and are worthy of future study.

L-655,708 prevented memory impairment without altering performance in oxygen-treated controls. Inverse agonists, including L-655,708, have a negative and opposite effect to that of classic agonists (*e.g.*, midazolam). Inverse agonists decrease channel opening whereas benzodiazepine agonists enhance channel opening.[21,33] The dose of L-665,708 used in the present study was carefully selected to preferentially inhibit α5GABA$_A$ receptors. *In vitro* electrophysiological and biochemical studies have confirmed that L-655,708 has a preference for α5GABA$_A$ receptors that is 107-, 61-, and 54-fold greater than GABA$_A$ receptors containing the α1, α2, and α3 subunits, respectively.[18] In addition, L-655,708, preferentially inhibits α5GABA$_A$ receptor–mediated currents in the hippocampus.[34] L-655,708, administered intraperitoneally at a dose of 1 mg/kg has previously been shown to yield 64% receptor occupancy of the α5GABA$_A$ receptor, but only 18% occupancy of α1, α2, and α3 subunit-containing GABA$_A$ receptors.[33] L-665,708 rapidly achieves its rapid peak dose ($t = 0.25$ h)[21] and has a relatively short half-life (0.5 h), despite a low plasma clearance rate (19 ml/kg in a rat model). After subcutaneous injection, concentrations in the brain mimic those in the plasma, indicating no tendency for L-655,708 to remain in the brain.[21] Thus, we assumed that L-655,705 was cleared 24 h after administration. L-655,708 likely prevents GABA$_A$ receptor activation during initial isoflurane exposure. Consistent with this notion are the results of a previous study that showed L-655,708 acts on α5GABA$_A$ receptors to prevent memory blockade by etomidate.[9] The memory-protective effect of L-655,708 is attributed to reduced α5GABA$_A$ receptor activity, although effects on other GABA$_A$ receptor subtypes cannot be entirely ruled out.

Fear-conditioning studies offer temporal resolution that can distinguish between short- and long-term memory.[35] In our study, short-term memory was more strongly affected by pretreatment with isoflurane than long-term memory, particularly when subjects were allowed 24 h to recover from anesthesia before fear-conditioning training. These results are interesting, given that the mechanisms involved in short- and long-term memory are increasingly understood as molecularly distinct processes.[12,35–39] Persistent memory-impairing effects of isoflurane may be mediated by alterations in the early phases of plasticity—in line with recent evidence from mouse slice preparations.[9] Isoflurane blocks the induction phase of long-term potentiation, an effect that can be reversed by inhibiting GABA$_A$ receptors with the competitive antagonist bicuculline.[40] Likewise, increased activity of α5GABA$_A$ receptors by etomidate prevents the induction of long-term potentiation in the hippocampus, an effect that can be reversed by L-655,708.[9] Long-term memory is strongly correlated with the maintenance of long-term potentiation and protein synthesis processes that underlie long-lasting plasticity.[41] The behavioral data presented in this study suggest the underlying mechanisms of long-term memory may be less liable to isoflurane anesthesia. Still, inhaled anesthetics are known to modify immediate-early gene transcription in response to early learning events.[32,42] Whether L-655,708 reverses the effects of isoflurane on synaptic plasticity and protein translation remains to be determined.

There are several potential limitations to the current study. There is a possibility that an unknown fraction of isoflurane was lost from the brain during transfer to closed tetrafluoroethylene syringes. However, others have used similar methods to measure and compare isoflurane concentrations in the brain and blood of rabbits.[25] Two rates of clearance for inhaled anesthetics have been observed, one from blood and the other from tissue, especially among those with high fat content.[28] After 270 min of elimination after isoflurane (1.3% for 90 min), 96% of the isoflurane had left the brain.[28] Furthermore, the blood and brain results were comparable after 30 and 90 min and indicated that negligible amounts of anesthetic were lost from brain samples.[28] Thus, we expect that the concentrations of isoflurane measured in the current study accurately reflect brain concentrations.

It should be noted that the choice of *post hoc* statistical testing may affect data interpretation. The LSD *post hoc* test was used in this study; however, after a statistical evaluation, the data were reanalyzed with the more conservative Tukey Honestly Significant Difference (HSD) test for unequal sample sizes. Results of the LSD and HSD *post hoc* analyses were compared. The LSD test revealed certain significant differences that were not detected with the HSD test. For example, although the LSD *post hoc* test detected significant long-term memory deficits in mice that were fear conditioned 1 h after isoflurane anesthesia ($P = 0.02$), the HSD test did not reveal this difference ($P = 0.17$). Although changes cannot be made to predetermined statistical methods after examination of the data, because the results of the LSD test and the more conservative HSD *post hoc* test differed, exact *P* values have been reported throughout the manuscript.

Postanesthesia memory deficits among human and animal subjects likely differ in time course and severity. This difference is partly the result of allometric scaling and pharmacokinetics. Indeed, mice ambulate within minutes after terminating the anesthetic, suggesting a more rapid recovery time compared with humans. In addition, the management of anesthesia in clinical practice and animal studies differs in terms of monitoring, the impact of noxious stimuli, and the strict management of hemodynamic and biochemical parameters.[43] Surgery and inflammation could exacerbate the

severity or extend the time course of anesthesia-related memory deficits.[44] The current results should prompt clinical studies to determine the incidence, severity, and functional impact of memory deficits in the early-postanesthesia period.

Among elderly patients, the incidence of postoperative cognitive dysfunction 24 h after sevoflurane anesthesia for minor surgery has been estimated to be as high as 47%.[4] In addition, patients who undergo isoflurane or propofol anesthesia for interventional neuroradiological procedures have shown memory decline for up to 24 h relative to their preoperative performance.[45] The functional consequence of such deficits is unknown but may have practical significance. Patients may need to have explicit recall for important information or undertake cognitively demanding tasks soon after their procedures. In particular, patients who undergo only brief diagnostic or surgical procedures typically expect to recover their baseline level of memory the day after anesthesia. Because inhibiting $\alpha5GABA_A$ receptors could promote intraoperative awareness, it is of interest to determine whether inverse agonists can treat—as well as prevent—memory deficits in the early postanesthesia period. Older generations of nonselective inverse agonists such as the β-carboline, methyl-6,7-dimethoxy-4-ethyl-β -carboline-3-carboxylate, and FG 7142 (ZK-31906) improve memory performance; however, these agents have epileptogenic and anxiogenic properties.[46] L-655,708 and orally administered, selective inverse agonists selective for the $\alpha5GABA_A$ receptor were not convulsant, proconvulsant, or anxiogenic in animal studies.[33,47] In human volunteers, pretreatment with an inverse agonist for $\alpha5GABA_A$ receptors, $\alpha5$IA, reversed memory impairment for word-list learning after the ingestion of ethanol.[47–50] Animal studies are currently underway to determine whether the administration of L-655,708 after anesthesia and fear conditioning can rescue, as well as prevent, memory deficits in the early postanesthesia period.

The authors thank James M. Sonner, M.D., Professor, Department of Anesthesia and Perioperative Care, University of California, San Francisco, San Francisco, California, for previewing the manuscript and for gas chromatographic analysis of isoflurane concentrations. They also thank Ella Czerwinska, M.Sc., Technician, University of Toronto, Toronto, Ontario, Canada, for technical support.

References

1. Johnson T, Monk T, Rasmussen LS, Abildstrom H, Houx P, Korttila K, Kuipers HM, Hanning CD, Siersma VD, Kristensen D, Canet J, Ibañaz MT, Moller JT, ISPOCD2 Investigators: 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, Fraidakis 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. Newman MF, Kirchner JL, Phillips-Bute B, Gaver V, Grocott H, Jones RH, Mark DB, Reves JG, Blumenthal JA, Neurological Outcome Research Group and the Cardiothoracic Anesthesiology Research Endeavors Investigators: Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. N Engl J Med 2001; 344: 395–402
4. Rohan D, Buggy DJ, Crowley S, Ling FK, Gallagher H, Regan C, Moriarty DC: Increased incidence of postoperative cognitive dysfunction 24 hr after minor surgery in the elderly. Can J Anaesth 2005; 52:137–42
5. Monk TG, Weldon BC, Garvan CW, Dede DE, van der Aa MT, Heilman KM, Gravenstein JS: Predictors of cognitive dysfunction after major noncardiac surgery. ANESTHESIOLOGY 2008; 108:18–30
6. 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
7. Culley DJ, Baxter MG, Yukhananov R, Crosby G: Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. ANESTHESIOLOGY 2004; 100:309–14
8. Kandel ER: The biology of memory: A forty-year perspective. J Neurosci 2009; 29:12748–56
9. Martin LJ, Zurek AA, MacDonald JF, Roder JC, Jackson MF, Orser BA: Alpha5GABAA receptor activity sets the threshold for long-term potentiation and constrains hippocampus-dependent memory. J Neurosci 2010; 30:5269–82
10. Barondes SH, Cohen HD: Memory impairment after subcutaneous injection of acetoxycycloheximide. Science 1968; 160:556–7
11. Izquierdo LA, Barros DM, Vianna MR, Coitinho A, deDavid e Silva T, Choi H, Moletta B, Medina JH, Izquierdo I: Molecular pharmacological dissection of short- and long-term memory. Cell Mol Neurobiol 2002; 22:269–87
12. Tully T, Preat T, Boynton SC, Del Vecchio M: Genetic dissection of consolidated memory in *Drosophila.* Cell 1994; 79:35–47
13. Hemmings HC Jr., Akabas MH, Goldstein PA, Trudell JR, Orser BA, Harrison NL: Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci 2005; 26:503–10
14. Caraiscos VB, Elliott EM, You-Ten KE, Cheng VY, Belelli D, Newell JG, Jackson MF, Lambert JJ, Rosahl TW, Wafford KA, MacDonald JF, Orser BA: Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. Proc Natl Acad Sci USA 2004; 101:3662–7
15. Hanning CD: Postoperative cognitive dysfunction. Br J Anaesth 2005; 95:82–7
16. Cheng VY, Martin LJ, Elliott EM, Kim JH, Mount HT, Taverna FA, Roder JC, Macdonald JF, Bhambri A, Collinson N, Wafford KA, Orser BA: Alpha5GABAA receptors mediate the amnestic but not sedative-hypnotic effects of the general anesthetic etomidate. J Neurosci 2006; 26:3713–20
17. Martin LJ, Oh GH, Orser BA: Etomidate targets alpha5 gamma-aminobutyric acid subtype A receptors to regulate synaptic plasticity and memory blockade. ANESTHESIOLOGY 2009; 111:1025–35
18. Quirk K, Blurton P, Fletcher S, Leeson P, Tang F, Mellilo D, Ragan CI, McKernan RM: [3H]L-655,708, a novel ligand selective for the benzodiazepine site of GABAA receptors which contain the alpha 5 subunit. Neuropharmacology 1996; 35:1331–5
19. Bailey KR, Rustay NR, Crawley JN: Behavioral phenotyping of transgenic and knockout mice: Practical concerns and potential pitfalls. Ilar J 2006; 47:24–31
20. Sonner JM, Gong D, Eger EI 2nd: Naturally occurring variability in anesthetic potency among inbred mouse strains. Anesth Analg 2000; 91:720–6
21. Atack JR, Pike A, Clarke A, Cook SM, Sohal B, McKernan RM, Dawson GR: Rat pharmacokinetics and pharmacodynamics of a sustained release formulation of the GABAA

alpha5-selective compound L-655,708. Drug Metab Dispos 2006; 34:887–93

22. Homanics GE, Ferguson C, Quinlan JJ, Daggett J, Snyder K, Lagenaur C, Mi ZP, Wang XH, Grayson DR, Firestone LL: Gene knockout of the alpha6 subunit of the gamma-aminobutyric acid type A receptor: Lack of effect on responses to ethanol, pentobarbital, and general anesthetics. Mol Pharmacol 1997; 51:588–96
23. Joo DT, Gong D, Sonner JM, Jia Z, MacDonald JF, Eger EI 2nd, Orser BA: Blockade of AMPA receptors and volatile anesthetics: Reduced anesthetic requirements in GluR2 null mutant mice for loss of the righting reflex and antinociception but not minimum alveolar concentration. ANESTHESIOLOGY 2001; 94:478–88
24. Mihic SJ, McQuilkin SJ, Eger EI 2nd, Ionescu P, Harris RA: Potentiation of gamma-aminobutyric acid type A receptor-mediated chloride currents by novel halogenated compounds correlates with their abilities to induce general anesthesia. Mol Pharmacol 1994; 46:851–7
25. Strum DP, Johnson BH, Eger EI 2nd: Elimination of anesthetics from rabbit brain. Science 1986; 234:1586–8
26. Liu J, Laster MJ, Taheri S, Eger EI 2nd, Chortkoff B, Halsey MJ: Effect of n-alkane kinetics in rats on potency estimations and the Meyer-Overton hypothesis. Anesth Analg 1994; 79:1049–55
27. Kamprath K, Wotjak CT: Nonassociative learning processes determine expression and extinction of conditioned fear in mice. Learn Mem 2004; 11:770–86
28. Wyrwicz AM, Conboy CB, Ryback KR, Nichols BG, Eisele P: In vivo 19F-NMR study of isoflurane elimination from brain. Biochim Biophys Acta 1987; 927:86–91
29. Bekker A, Shah R, Quartermain D, Li YS, Blanck T: Isoflurane preserves spatial working memory in adult mice after moderate hypoxia. Anesth Analg 2006; 102:1134–8
30. Holaday DA, Fiserova-Bergerova V, Latto IP, Zumbiel MA: Resistance of isoflurane to biotransformation in man. ANESTHESIOLOGY 1975; 43:325–32
31. Rau V, Oh I, Laster M, Eger EI 2nd, Fanselow MS: Isoflurane suppresses stress-enhanced fear learning in a rodent model of post-traumatic stress disorder. ANESTHESIOLOGY 2009; 110:487–95
32. Alkire MT, Gorski LA: Relative amnesic potency of five inhalational anesthetics follows the Meyer-Overton rule. ANESTHESIOLOGY 2004; 101:417–29
33. Atack JR, Bayley PJ, Seabrook GR, Wafford KA, McKernan RM, Dawson GR: L-655,708 enhances cognition in rats but is not proconvulsant at a dose selective for alpha5-containing GABAA receptors. Neuropharmacology 2006; 51:1023–9
34. Casula MA, Bromidge FA, Pillai GV, Wingrove PB, Martin K, Maubach K, Seabrook GR, Whiting PJ, Hadingham KL: Identification of amino acid residues responsible for the alpha5 subunit binding selectivity of L-655,708, a benzodiazepine binding site ligand at the GABA(A) receptor. J Neurochem 2001; 77:445–51
35. Schafe GE, Nadel NV, Sullivan GM, Harris A, LeDoux JE: Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. Learn Mem 1999; 6:97–110
36. Barondes SH: Cerebral protein synthesis inhibitors block long-term memory. Int Rev Neurobiol 1970; 12:177–205
37. Peters M, Bletsch M, Catapano R, Zhang X, Tully T, Bourtchouladze R: RNA interference in hippocampus demonstrates opposing roles for CREB and PP1alpha in contextual and temporal long-term memory. Genes Brain Behav 2009; 8:320–9
38. Saab BJ, Georgiou J, Nath A, Lee FJ, Wang M, Michalon A, Liu F, Mansuy IM, Roder JC: NCS-1 in the dentate gyrus promotes exploration, synaptic plasticity, and rapid acquisition of spatial memory. Neuron 2009; 63:643–56
39. Yin JC, Tully T: CREB and the formation of long-term memory. Curr Opin Neurobiol 1996; 6:264–8
40. Simon W, Hapfelmeier G, Kochs E, Zieglgänsberger W, Rammes G: Isoflurane blocks synaptic plasticity in the mouse hippocampus. ANESTHESIOLOGY 2001; 94:1058–65
41. Nguyen PV, Woo NH: Regulation of hippocampal synaptic plasticity by cyclic AMP-dependent protein kinases. Prog Neurobiol 2003; 71:401–37
42. Ren Y, Zhang FJ, Xue QS, Zhao X, Yu BW: Bilateral inhibition of gamma-aminobutyric acid type A receptor function within the basolateral amygdala blocked propofol-induced amnesia and activity-regulated cytoskeletal protein expression inhibition in the hippocampus. ANESTHESIOLOGY 2008; 109:775–81
43. Loepke AW, McGowan FX Jr., Soriano SG: CON: The toxic effects of anesthetics in the developing brain: The clinical perspective. Anesth Analg 2008; 106:1664–9
44. Wan Y, Xu J, Ma D, Zeng Y, Cibelli M, Maze M: Postoperative impairment of cognitive function in rats: A possible role for cytokine-mediated inflammation in the hippocampus. ANESTHESIOLOGY 2007; 106:436–43
45. Münte S, Münte TF, Kuche H, Osthaus A, Herzog T, Heine J, Leuwer M, Piepenbrock S: General anesthesia for interventional neuroradiology: Propofol *versus* isoflurane. J Clin Anesth 2001; 13:186–92
46. Möhler H, Rudolph U, Boison D, Singer P, Feldon J, Yee BK: Regulation of cognition and symptoms of psychosis: Focus on GABA(A) receptors and glycine transporter 1. Pharmacol Biochem Behav 2008; 90:58–64
47. Chambers MS, Atack JR, Carling RW, Collinson N, Cook SM, Dawson GR, Ferris P, Hobbs SC, O'connor D, Marshall G, Rycroft W, Macleod AM: An orally bioavailable, functionally selective inverse agonist at the benzodiazepine site of GABAA alpha5 receptors with cognition enhancing properties. J Med Chem 2004; 47:5829–32
48. Chambers MS, Atack JR, Broughton HB, Collinson N, Cook S, Dawson GR, Hobbs SC, Marshall G, Maubach KA, Pillai GV, Reeve AJ, MacLeod AM: Identification of a novel, selective GABA(A) alpha5 receptor inverse agonist which enhances cognition. J Med Chem 2003; 46:2227–40
49. Dawson GR, Maubach KA, Collinson N, Cobain M, Everitt BJ, MacLeod AM, Choudhury HI, McDonald LM, Pillai G, Rycroft W, Smith AJ, Sternfeld F, Tattersall FD, Wafford KA, Reynolds DS, Seabrook GR, Atack JR: An inverse agonist selective for alpha5 subunit-containing GABAA receptors enhances cognition. J Pharmacol Exp Ther 2006; 316:1335–45
50. Nutt DJ, Besson M, Wilson SJ, Dawson GR, Lingford-Hughes AR: Blockade of alcohol's amnestic activity in humans by an alpha5 subtype benzodiazepine receptor inverse agonist. Neuropharmacology 2007; 53:810–20