

Effect of Isoflurane and Other Potent Inhaled Anesthetics on Minimum Alveolar Concentration, Learning, and the Righting Reflex in Mice Engineered to Express α_1 γ-Aminobutyric Acid Type A Receptors Unresponsive to Isoflurane

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Background: **Enhancement of the function of γ-aminobutyric acid type A receptors containing the α_1 subunit may underlie a portion of inhaled anesthetic action. To test this, the authors created gene knock-in mice harboring mutations that render the receptors insensitive to isoflurane while preserving sensitivity to halothane.**

Methods: **The authors recorded miniature inhibitory synaptic currents in hippocampal neurons from hippocampal slices from knock-in and wild-type mice. They also determined the minimum alveolar concentration (MAC), and the concentration at which 50% of animals lost their righting reflexes and which suppressed pavlovian fear conditioning to tone and context in both genotypes.**

Results: **Miniature inhibitory postsynaptic currents decayed more rapidly in interneurons and CA1 pyramidal cells from the knock-in mice compared with wild-type animals. Isoflurane (0.5–1 MAC) prolonged the decay phase of miniature inhibitory postsynaptic currents in neurons of the wild-type mice, but this effect was significantly reduced in neurons from knock-in mice. Halothane (1 MAC) slowed the decay of miniature inhibitory postsynaptic current in both genotypes. The homozygous knock-in mice were more resistant than wild-type controls to loss of righting reflexes induced by isoflurane and enflurane, but not to halothane. The MAC for isoflurane, desflurane, and halothane did not differ between knock-in and wild-type mice. The knock-in mice and wild-type mice did not differ in their sensitivity to isoflurane for fear conditioning.**

Conclusions: **γ-Aminobutyric acid type A receptors containing the α_1 subunit participate in the inhibition of the righting reflexes by isoflurane and enflurane. They are not, however, involved in the amnestic effect of isoflurane or immobilizing actions of inhaled agents.**

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

ALL inhaled anesthetics supply two essential elements of anesthesia: immobility and amnesia. A current consensus argues that these elements result from the combined effects of inhaled anesthetics on several ligand-gated and voltage-gated channels.[1] The γ-aminobutyric acid type A receptors ($GABA_A$Rs) have been considered prime candidates as targets of inhaled anesthetic action[2] because they are widely distributed in the central nervous system and because many inhaled agents promote their function at clinically relevant concentrations. In fact, enhancement of $GABA_A$R function seems to underlie the production of anesthesia by the intravenous anesthetics propofol and etomidate, which have effects on $GABA_A$R that are similar to those of inhaled anesthetics.[3] Many inhaled anesthetics similarly prolong $GABA_A$R-mediated inhibition of spinal motoneurons,[4] which may account in part for the immobilizing effect of inhaled anesthetics, and enhance inhibition in the hippocampus,[5] which might mediate amnestic effects. However, recent studies suggest that $GABA_A$Rs might not be important mediators of immobility. For example, although xenon, cyclopropane, and isoflurane differ greatly in their capacity to enhance the $GABA_A$R response to GABA, the intrathecal administration of the $GABA_A$R antagonist, picrotoxin, produces a modest increase in minimum alveolar concentration (MAC) that does not differ among these anesthetics.[6]

Our group has developed a strain of knock-in mouse to test the hypothesis that $GABA_A$Rs containing the α_1 subunit mediate some or all of the clinically important behavioral effects of inhaled volatile anesthetics such as isoflurane. The α_1 subunit is the most abundant of the $GABA_A$R α subunits, being present in approximately 40% of all $GABA_A$ receptors in the brain,[7] with a relatively ubiquitous distribution throughout the cerebral cortex, thalamus, cerebellum, and hippocampus. The mice were genetically engineered to express two point mutations in the GABRA1 locus that changes serine (S) to histidine (H) at position 270 and leucine (L) to alanine (A) at position 277 in the α_1 subunit polypeptide. *In vitro*, the S270H mutation selectively eliminates $GABA_A$R potenti-

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ation by isoflurane and desflurane, but not halothane. Incorporation of the second L277A mutation restores the GABA sensitivity of the mutant receptor to normal.[8,9]

If $GABA_AR$-containing α_1 subunits mediate any of the behavioral effects of the inhaled anesthetics, these knock-in mice should have a reduced sensitivity to isoflurane (but not halothane), relative to wild-type controls. We used three standard behavioral tests: limb withdrawal in response to noxious stimulation (*i.e.*, measurement of "MAC"), acquisition of fear conditioning to tone and to context (*i.e.*, pavlovian conditioning), and loss of the righting reflex (LORR), measures widely used in studies of anesthetic effects in rodents.

Materials and Methods

Experimental Subjects

Institutional animal care and use committees (University of California, San Francisco, California, and University of Pittsburgh, Pittsburgh, Pennsylvania) approved our studies of male and female mice bred from mice heterozygous for the S270H and L277A mutations, producing wild-type (SL/SL), heterozygous knock-in (SL/HA), and homozygous knock-in (HA/HA) mice. Animals were housed under a 12-h light-and-dark cycle and had continuous access to standard mouse chow and tap water.

Hippocampal Slice Electrophysiology

Brain slices were prepared from adult mice (age range, 28–52 days), and miniature inhibitory postsynaptic currents (mIPSCs) were recorded and analyzed as previously described.[10] Interneurons and pyramidal cells in the CA1 stratum radiatum and stratum lacunosum-moleculare layer of the hippocampus were identified using differential interference contrast microscopy.[10] The identity of pyramidal cells was then verified by pronounced accommodation of action potential firing, to distinguish them from interneurons also present in the pyramidal cell layer.[11] As in a previous study,[10] fewer than 10% of the recorded neurons in the CA1 pyramidal cell layer were interneurons. Whole cell patch clamp recordings were obtained with borosilicate glass pipettes that had a resistance of 2–4 MΩ when filled with pipette solution containing 130 mM cesium methanesulfonate (CH_3SO_3Cs), 8.3 mM sodium methanesulfonate (CH_3SO_3Na), 1.7 mM NaCl, 1 mM $CaCl_2$, 10 mM EGTA (intracellular free calcium ion concentration: approximately 0.01 μM), 2 mM Mg-ATP, 0.3 mM Na-GTP, and 10 mM HEPES (pH 7.2, osmolarity 295 mOsm). In the recording chamber, brain slices were constantly superfused at a rate of 3.5 ml/min with artificial cerebrospinal fluid containing 117 mM NaCl, 3.6 mM KCl, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, and 11 mM glucose (pH 7.4, osmolarity 305 mOsm). To eliminate action potential–evoked synaptic events, 0.5 μM tetrodotoxin was added to the artificial cerebrospinal fluid. Drugs were applied *via* a gravitational glass-syringe/polytetrafluoroethylene-tube system to reduce the loss of volatile anesthetics. A 10-min preapplication of each drug was performed before the 5-min data recording epoch that was used for analysis; this ensured that the anesthetic drugs were at equilibrium throughout the data collection period.[12] The mIPSCs were recorded at room temperature for 5 min with an acquisition frequency of 10 kHz and a filter frequency of 2 kHz. The holding potential was −60 mV. Analysis of the mIPSC data traces was performed as described previously.[10] Bath application of 20 μM bicuculline blocked all mIPSC activity (n = 3, data not shown). The weighted decay time constant (τ_{decay}) was calculated as $\tau_{decay} = (A_f \times t_f + A_s \times t_s)/(A_f + A_s)$.[10] To ensure an adequate recording configuration throughout the experiments, we determined the access resistance before and after each recording using the membrane properties feature of the acquisition software (pClamp 9.0; Axon Instruments, Foster City, CA). Recordings were excluded from the analysis when the difference between membrane and access resistance decreased below a 10-fold value.

Volatile anesthetic solutions were prepared in airtight surgical bags. Each bag contained 100 ml artificial cerebrospinal fluid including 0.5 μM tetrodotoxin plus volatile anesthetic, as described previously. The values obtained for amplitudes and τ_{decay} were compared to the respective control values in volatile anesthetic–free solution to calculate the volatile anesthetic effect on mIPSCs as percentage change for each experiment, and comparisons between genotypes were made using analysis of variance.[10]

Whole-animal Behavioral Studies

Studies of learning and memory applied techniques described previously.[13–15] Mice (n = 254) were exposed to a target concentration of isoflurane (confirmed with gas chromatography) for 30 min before training. Each animal was then rapidly transferred to a training chamber containing the target concentration of isoflurane and was allowed to explore the chamber for 3 min before training began. Mice then received three tone-shock pairs (tone training) consisting of a 30-s tone (90 dB, A-scale, 2,000 Hz) coterminating with a 2-s electric shock (11-Hz bipolar square waves). Ninety seconds separated tone-shock pairs. Animals were returned to their home cages within 60 s after the last shock. The shock currents were 2 mA at 0, 0.1%, 0.2%, 0.3%, and 0.4% isoflurane. At 0.5% and 0.75% isoflurane, we used 3-mA currents. For each test, the anesthetic concentration was calculated as the mean of the concentrations measured in the training chambers before and after training of that set of four mice.

Both context and tone testing took place the day after training. For tone testing, each animal was placed in a

special test chamber and, after 3 min of exploration, a tone (90 dB, A-scale, 2,000 Hz) was continuously sounded for 8 min; no shock was administered. Context testing was conducted 1–2 h later. For context testing, the mice were returned to the chambers used to supply the electric shocks, whereas tone testing took place in chambers providing an entirely different environment from that provided by the training chambers. For both context and tone testing, four mice were observed simultaneously, one in each of four separate test chambers, *via* a video camera. No personnel were in the tone or context training or testing rooms during training or testing. To score freezing to either tone or context, an observation of one of the four animals was made every 2 s. Therefore, each animal was scored once every 8 s. Behavior was judged as freezing if there was no visible movement except for breathing.[16] The observation periods were video-recorded for scoring by a blinded observer.

The percentage of time an animal froze during the 8-min observation periods was calculated as the number of observations judged to be freezing divided by the total number of observations in 8 min, *i.e.*, 60 observations.[16] For each group score at a given isoflurane concentration, the mean freeze score and standard error of the mean (SE) were calculated.

A least-squares linear regression was applied to the raw data for fear to context for 0–0.5% isoflurane. For fear to tone data, a least-squares linear regression was applied to the data for 0–0.75% isoflurane. The concentration (EC_{50}) producing a 50% decrease in freezing scores from control (no isoflurane) and the SE of this concentration were calculated and used to compare freezing to context and freezing to tone for the three genetic groups. In addition, two-way analysis of variance using genotype and anesthetic concentration as factors was performed to determine whether there was a difference in the effect of genotype on freeze scores. A value of $P < 0.05$ was regarded as significant for all comparisons.

We measured MAC for desflurane, isoflurane, and halothane in 88 mice (28 SL/SL, 36 SL/HA, and 24 HA/HA) as described previously.[17] These mice were a random subset of mice after measurements of fear conditioning had been made. Each mouse was used as a subject for one, two, or three of the test anesthetics but was used only once for a test of a given anesthetic. For each mouse, MAC was calculated as the mean of the greatest inspired concentration that permitted movement in response to tail clamp and the smallest concentration that prevented movement. Genotyping of each test mouse was accomplished after the determinations of learning and memory, and MAC. Differences in MAC between genotypes were determined using analysis of variance.

Adult (8- to 12-week-old) male and female mice (n = 15–18 per genotype) were tested for sensitivity to inhaled anesthetics using the LORR assay as described.[18,19] Briefly, mice were placed in individual wire mesh cages in a rotating carousel in a sealed acrylic chamber and anesthetized. Within the chamber, carbon dioxide was maintained at less than 1% atm, and temperature was maintained at 35° ± 0.2°C. Chamber atmosphere and anesthetic concentrations were monitored continuously with a Datex Capnomac Ultima device (Datex-Ohmeda, Helsinki, Finland), and fresh oxygen was delivered at a rate of 1.5 l/min. Mice were equilibrated with the desired concentration (% atm) of halothane (Halocarbon Laboratories, River Edge, NJ), isoflurane (Halocarbon Laboratories), or enflurane (Anaquest, Madison, WI) for 15 min, after which they were scored by an observer blind to the genotypes. Scores were quantal; a positive response for LORR occurred when mice were not able to right themselves two times during five revolutions of the carousel at 4 rpm. Mice were allowed to recuperate in oxygen for at least 20 min before being equilibrated with the next anesthetic concentration. The dose–response relation for each anesthetic was analyzed using the Z statistic.[20]

Results

Hippocampal Slice Electrophysiology

Miniature inhibitory postsynaptic currents were recorded from interneurons (n = 36) and pyramidal cells (n = 23) in the CA1 subfield of the hippocampus. The cell properties (input resistance, cell capacitance) were not significantly different between genotypes in interneurons and pyramidal cells, and there was also no significant difference between the groups of interneurons and pyramidal cells. In interneurons, the decay phase of mIPSCs recorded in neurons from HA/HA animals was significantly faster compared with mIPSCs recorded in SL/SL and SL/HA animals (figs. 1A and B). In pyramidal cells, we observed a significant faster decay time constant in HA/HA animals compared with SL/SL animals (fig. 1B). However, mIPSC amplitude and frequency were not significantly different between the genotypes (data not shown).

Bath application of isoflurane (0.16 and 0.31 mM; 1 MAC corresponds to 0.31 mM) prolonged the decay phase of mIPSCs in SL/SL animals, and this effect was concentration dependent. The effect of isoflurane was significantly reduced in HA/HA animals compared with wild-type animals as shown in example current traces in figure 1C. Halothane application (0.27 mM, the aqueous concentration at 1 MAC) significantly prolonged the decay time of mIPSCs in SL/SL animals and in the mutant genotypes, with no significant differences (fig. 1D). To determine the average effect of the volatiles on the decay time constants, we calculated the percentage change between control conditions and after 10 min of volatile application. The average data are shown in two bar diagrams in figures 2A and B. We found that the effect of isoflurane on mIPSC decay times was reduced in inter-

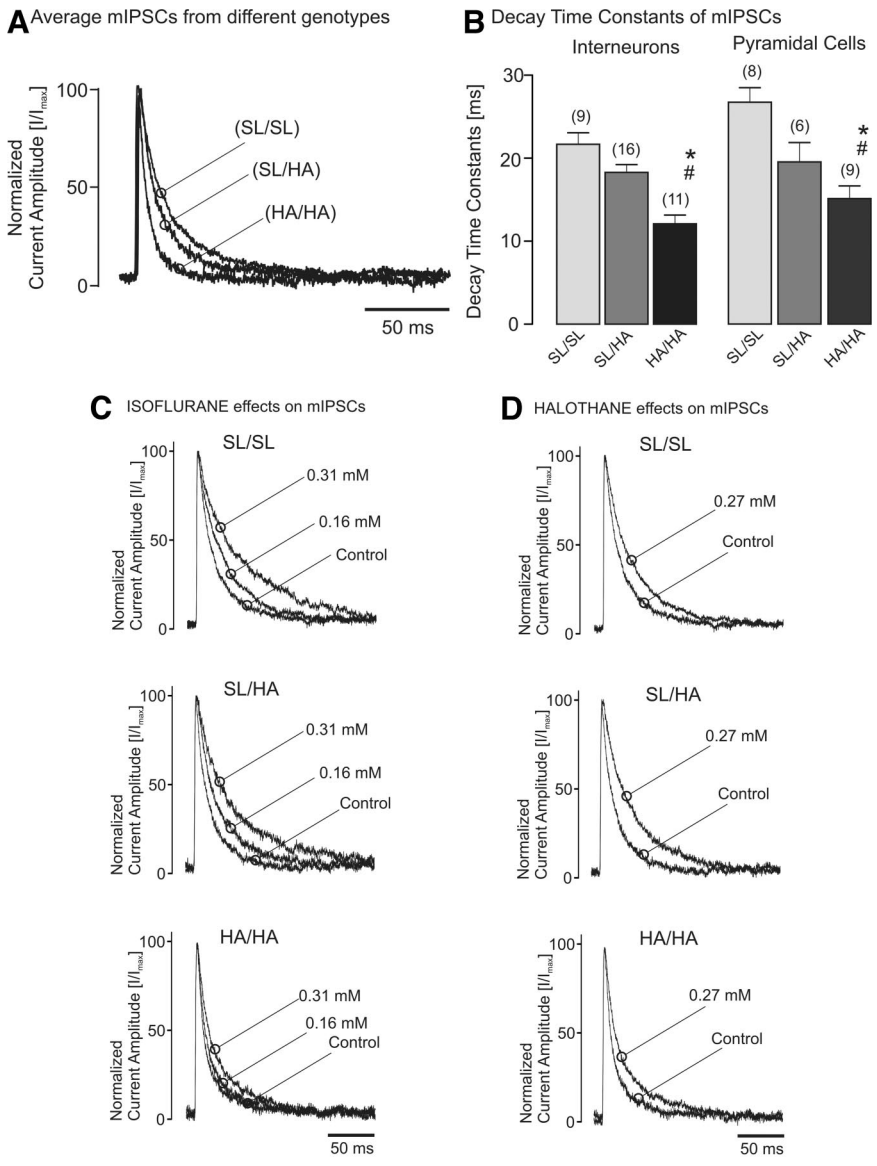

Fig. 1. Example traces of miniature postsynaptic currents (mIPSCs) under control conditions and during application of volatile anesthetics. (*A*) Average mIPSC traces (from 50 single events) of hippocampal interneurons from the three genotypes (wild-type SL/SL; heterozygous SL/HA; homozygous HA/HA) under control conditions. The current amplitude is normalized to the maximum amplitude to visually compare the decay time of the mIPSCs. (*B*) Bar diagram of average decay time constants from interneurons and pyramidal cells for all three genotypes. Number of experiments is given in brackets, and significance is indicated at the $P < 0.05$ level (* significantly different from SL/SL; # significantly different from SL/HA). (*C*) Effect of 0.16 and 0.31 mM isoflurane on average current traces in all three genotypes. (*D*) Effect of 0.27 mM halothane on average current traces in all three genotypes.

neurons and pyramidal cells (figs. 2A and B). The amplitudes and frequencies of mIPSCs were not significantly affected by volatile anesthetic application in any of the three genotypes (data not shown).

Behavioral Studies

Mice were tested for the amnestic effects of inhaled anesthetics using a fear conditioning assay. Control values for fear to context and fear to tone (*i.e.*, in the absence of anesthetic) did not differ among the three genotypes of mice. Slopes and intercepts did not differ by genotype among mice conditioned to context or among those conditioned to tone (figs. 3 and 4). Mice of different genotypes did not differ in conditioning to tone ($F_{2,248} = 0.11$, $P = 0.89$) or context ($F_{2,226} = 0.16$, $P = 0.85$). The $EC_{50} \pm$ SE (in % atm isoflurane) for freezing to tone was 0.34 ± 0.05 for SL/SL mice (n = 72), 0.34 ± 0.04 for SL/HA mice (n = 113 mice), and 0.31 ± 0.06 for HA/HA mice (n = 66). For freezing to context, these values were 0.28 ± 0.05 for SL/SL (n = 64), 0.25 ± 0.04 for SL/HA (n = 101), and 0.24 ± 0.06 for HA/HA (n = 64).

Mice were tested for the immobilizing effects of inhaled anesthetics in response to a noxious stimulus using the standard tail clamp/withdrawal assay. MAC values did not differ between genotypes for any anesthetic tested (table 1). Using one-way analysis of variance, for isoflurane, $F_{2,48} = 0.05$ ($P = 0.95$); for desflurane, $F_{2,32} = 1.32$ ($P = 0.29$); and for halothane, $F_{2,38} = 0.07$ ($P = 0.94$).

Mice were also tested for the motor ataxic effects of inhaled anesthetics using the standard LORR assay. In this assay, halothane EC_{50} values did not differ between SL/SL (0.68 ± 0.02) and HA/HA (0.69 ± 0.02) mice (fig. 5A). However, differences in the EC_{50} values were observed for isoflurane (0.63 ± 0.02 for SL/SL *vs.* 0.72 ± 0.02 for HA/HA; $P < 0.01$; fig. 5B) and enflurane (1.09 ± 0.03 for SL/SL *vs.* 1.21 ± 0.03 for HA/HA; $P < 0.001$; fig. 5C).

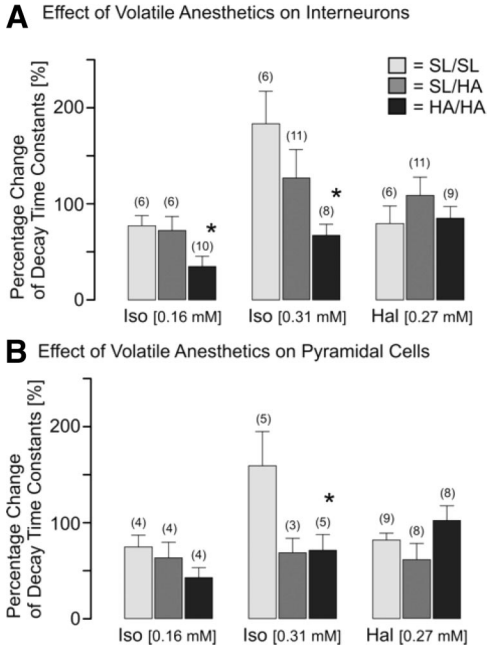

Fig. 2. Effect of volatile anesthetics on decay time constants of miniature postsynaptic currents in interneurons and pyramidal cells. (*A*) Bar diagram of volatile anesthetic–induced percentage change on decay time constants of miniature postsynaptic currents in interneurons for all three genotypes (wild-type SL/SL, heterozygous SL/HA, and homozygous HA/HA). Number of experiments is given in parentheses, and significance is indicated at the $P < 0.05$ level (* significantly different from SL/SL). (*B*) Bar diagram of volatile anesthetic–induced percentage change on decay time constants of miniature postsynaptic currents in pyramidal cells for all three genotypes. Number of experiments is given in parentheses, and significance is indicated at the $P < 0.05$ level (* significantly different from SL/SL).

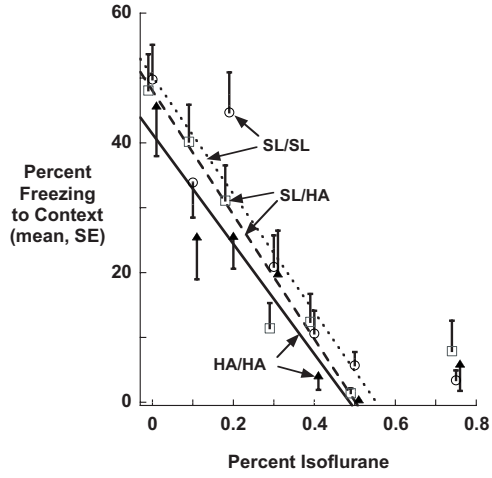

Fig. 3. Fear to context was measured as the percent of time a mouse froze in the presence of the environment in which the mouse had received three foot shocks on the preceding day. A greater percentage time spent freezing indicated greater remembrance. Increasing isoflurane concentration from 0 to 0.5% decreased freezing in a rectilinear manner. There was no difference by genotype in the capacity of isoflurane to interfere with fear to context as assessed by slope, intercept, or the effective concentration producing 50% of the maximal response (EC_{50}) of the regression lines. For SL/SL (wild-type) mice, the slope was −90.4 ± 12.2 and the intercept was 50.3 ± 3.6 (n = 64). For SL/HA (heterozygous) mice, the slope and intercept (± SE) were −95.8 ± 11.6 and 48.2 ± 3.4 (n = 101 mice). For HA/HA (homozygous knock-in) mice, these values were −85.1 ± 15.0 and 41.6 ± 4.2 (n = 64). Data for genotypes are displaced slightly on the abscissa to decrease overlapping of error bars.

Discussion

The knock-in mice harboring the HA/HA mutation were designed to enable us to evaluate the significance of the α_1 subunit to the anesthetic effects of isoflurane. The electrophysiologic recordings from hippocampal neurons show that the decay time constants of mIPSCs in interneurons from HA/HA mice decay significantly faster compared with the mIPSCs of wild-type mice (SL/SL). In addition, whereas isoflurane and halothane prolonged mIPSCs in the wild-type animals, the effects of isoflurane (but not halothane) was significantly reduced in the HA/HA animals. These findings are all consistent with the expression in the hippocampus of mutated α_1 subunits, and with a role for GABA$_A$Rs containing α_1 subunits in generating inhibitory synaptic currents in pyramidal cells and interneurons. The smaller residual effect of isoflurane in the HA/HA animals presumably reflects the activation of a distinct population of isoflurane-sensitive GABA$_A$Rs at hippocampal synapses, perhaps containing α_2 and/or α_3 subunits. At GABAergic synapses onto cerebellar Purkinje cells,[21] for example, or thalamocortical relay neurons,[22] the IPSC is generated by activation of a more homogeneous population of GABA$_A$Rs containing α_1 subunits, and IPSC kinetics are fast (τ = 5–10 ms). The kinetics of hippocampal IPSCs[10] are slower and presumably reflect a mixed population of receptors.

The mice harboring the α_1 subunit HA/HA mutation displayed normal sensitivity to the amnestic effects of isoflurane. This suggests that GABA$_A$ receptors containing the α_1 subunit do not mediate the capacity of isoflurane to produce amnesia. This is an interesting result, because GABA$_A$ receptors containing the α_1 subunit make up approximately half of the total receptor population in the rodent brain.[7] In addition, our hippocampal electrophysiology shows a decrease in the pharmacologic effect of the anesthetic *in vivo* in a structure known to be important for learning, the hippocampus. Clearly, such receptors are not critical to the amnestic effect of isoflurane, as they have been shown to be for benzodiazepines.[23] The amygdala is another structure known to be important for the acquisition of fear conditioning, and its circuitry is thought to use GABA$_A$Rs that contain other subunits, such as α_2 and α_5, that have been implicated in the anxiolytic and amnestic effects of the benzodiazepines.[23] Therefore, several factors limit the generalization of our results from the GABA$_A$R-containing α_1 subunits and isoflurane to other GABA$_A$R subtypes and anesthetics. In addition, the faster decay of IPSCs in the HA/HA mice compared with wild-type mice may also lead to compensation for the HA/HA mutation.

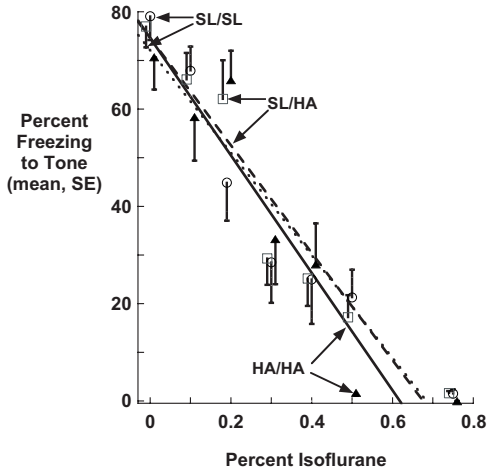

Fig. 4. Fear to tone was measured as the percent of time a mouse froze in the presence of the tone supplied previously (the day before). Freezing was tested in an environment that otherwise differed (in shape, texture and shape of ground and walls, smell, lighting) from the environment in which the mouse had been trained. A greater percentage time spent freezing indicated greater remembrance. Increasing isoflurane concentration from 0 to 0.75% decreased freezing in a rectilinear manner. There was no difference by genotype in the capacity of isoflurane to interfere with fear to context as assessed by slope, intercept, or the effective concentration producing 50% of the maximal response (EC_{50}) of the regression lines. For SL/SL (wild-type) mice, the intercept was −105.9 ± 10.9 and the slope was −105.9 ± 11.0, 72.4 ± 4.2 (n = 72). For SL/HA (heterozygous) mice, the slope and intercept (± SE) were −109.6 ± 9.0 and 74.6 ± 3.4 (n = 113 mice). For HA/HA (homozygous knock-in) mice, these values were −120.2 ± 17.6 and 74.7 ± 5.4 (n = 66). Data for genotypes are displaced slightly on the abscissa to decrease overlapping of error bars.

Although $GABA_A$Rs containing the α_1 subunit may not mediate the capacity of isoflurane to produce amnesia, this may not apply to other inhaled anesthetics.

We also conclude that $GABA_A$Rs containing the α_1 subunit do not mediate the capacity of desflurane, isoflurane, or halothane to produce immobility in the face of noxious stimulation ("MAC"). This result is less surprising in the sense that inhaled anesthetics are believed to act at the level of the spinal cord, where the $GABA_A$R α_1 subunit is not highly expressed, although immobility induced by the intravenous anesthetics propofol and etomidate is reduced by mutations in the $GABA_A$R β_3 subunit.[3] The current results for MAC are also consistent with results from pharmacologic studies that suggest no mediation by $GABA_A$R of the immobility produced by inhaled anesthetics.[6,24,25]

Table 1. Effect of the α_1 γ-Aminobutyric Acid Type A Receptor Knock-in Mutations on Minimum Alveolar Concentration

Genotype	n	Isoflurane MAC	n	Desflurane MAC	n	Halothane MAC
SL/SL	16	1.50 ± 0.20	13	7.61 ± 0.64	17	1.27 ± 0.15
SL/HA	19	1.45 ± 0.18	11	7.81 ± 0.58	15	1.26 ± 0.13
HA/HA	16	1.37 ± 0.14	11	7.38 ± 0.64	9	1.28 ± 0.08

Values are given as mean ± SD. HA/HA are homozygous knock-in mice. SL/SL are wild-type mice. SL/HA mice contain one knock-in and one wild-type allele of the α_1 γ-aminobutyric acid type A receptor. There were no significant differences by analysis of variance between genotypes for any anesthetic.

MAC = minimum alveolar concentration.

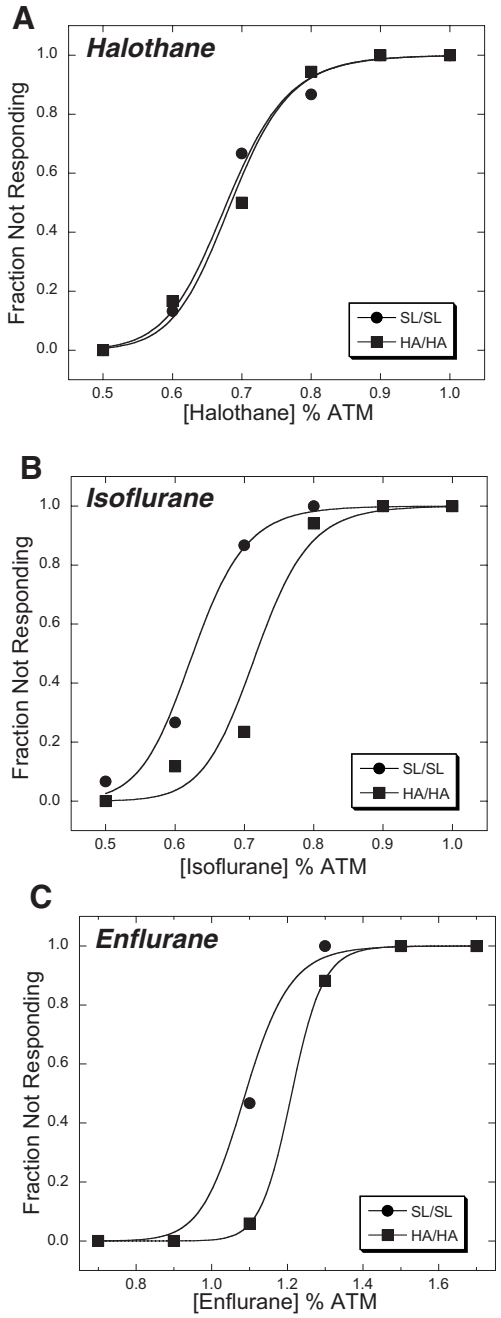

Fig. 5. (*A*) The concentration of halothane producing loss of righting reflexes did not differ between SL/SL (wild-type) and HA/HA (homozygous knock-in) mice. HA/HA mice inhaling isoflurane (*B*) or enflurane (*C*) required more anesthetic than SL/SL mice for loss of righting reflexes ($P < 0.01$ and $P < 0.001$, respectively).

The HA/HA mice showed an increase in the EC_{50} for isoflurane and enflurane, but not halothane in the LORR test. Because the $GABA_A$R containing the mutated α_1 subunit does not respond to isoflurane or enflurane, but retains sensitivity to halothane, this result provides evidence that the circuits involved in the LORR response

involve $GABA_A$Rs containing α_1 subunits. The LORR response produced by ethanol was not altered by this mutation[26]—this difference may reflect the much larger potentiation of $GABA_A$R function by anesthetic concentrations of isoflurane as compared with ethanol. The α_1 HA/HA mutation produces a 14% change in LORR for isoflurane, suggesting that other $GABA_A$R subtypes or additional anesthetic targets are important in generating LORR.

Taken together, these studies suggest the surprising conclusion that several behavioral actions of isoflurane do not require activation of $GABA_A$Rs containing α_1 subunits, despite the ubiquity of this receptor subtype at subsynaptic locations in a variety of cortical and subcortical regions, whereas LORR does indeed require $GABA_A$Rs containing α_1 subunits. Recent studies suggest that extrasynaptic $GABA_A$Rs, which can contain α_4, α_5, α_6, and/or δ subunits, may be especially sensitive to ethanol and volatile anesthetics,[27–30] and these may also prove to be significant for the behavioral actions of these drugs. Mice bearing mutations containing α_4, α_5, α_6, and/or δ subunits in these receptors that parallel those engineered for the current report for α_1 subunits will allow an evaluation of this hypothesis.

References

1. Sonner JM, Antognini JF, Dutton RC, Flood P, Gray AT, Harris RA, Homanics GE, Kendig J, Orser B, Raines DE, Rampil IJ, Trudell J, Vissel B, Eger EI II: Inhaled anesthetics and immobility: Mechanisms, mysteries, and minimum alveolar anesthetic concentration. Anesth Analg 2003; 97:718–40
2. Tanelian DL, Kosek P, Mody I, MacIver MB: The role of the $GABA_A$ receptor/chloride channel complex in anesthesia. ANESTHESIOLOGY 1993; 78:757–76
3. Jurd R, Arras M, Lambert S, Drexler B, Siegwart R, Crestani F, Zaugg M, Vogt KE, Ledermann B, Antkowiak B, Rudolph U: General anesthetic actions *in vivo* strongly attenuated by a point mutation in the $GABA_A$ receptor β3 subunit. FASEB J 2003; 17:250–2
4. Eccles JC, Schmidt R, Willis WD: Pharmacological studies on presynaptic inhibition. J Physiol 1963; 168:500–30
5. Pearce RA, Stringer JL, Lothman EW: Effect of volatile anesthetics on synaptic transmission in the rat hippocampus. ANESTHESIOLOGY 1989; 71:591–8
6. Zhang Y, Sonner JM, Eger EI II, Stabernack CR, Laster MJ, Raines DE, Harris RA: Gamma-aminobutyric $acid_A$ receptors do not mediate the immobility produced by isoflurane. Anesth Analg 2004; 99:85–90
7. McKernan RM, Whiting PJ: Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci 1996; 19:139–43
8. Topf N, Jenkins A, Baron N, Harrison NL: Effects of isoflurane on γ-aminobutyric acid type A receptors activated by full and partial agonists. ANESTHESIOLOGY 2003; 98:306–11
9. Borghese CM, Werner DF, Topf N, Baron NV, Henderson LA, Boehm SL II, Blednov YA, Saad A, Dai S, Pearce RA, Harris RA, Homanics GE, Harrison NL: An isoflurane- and alcohol-insensitive mutant $GABA_A$ receptor α1 subunit with near-normal apparent affinity for GABA: Characterization in heterologous systems and production of knockin mice. J Pharmacol Exp Ther 2006; 319:208–18
10. Goldstein PA, Elsen FP, Ying SW, Ferguson C, Homanics GE, Harrison NL: Prolongation of hippocampal miniature inhibitory postsynaptic currents in mice lacking the $GABA_A$ receptor α1 subunit. J Neurophysiol 2002; 88:3208–17
11. Nishikawa K, MacIver MB: Membrane and synaptic actions of halothane on rat hippocampal pyramidal neurons and inhibitory interneurons. J Neurosci 2000; 20:5915–23
12. Elsen FP, Liljelund P, Werner DF, Olsen RW, Homanics GE, Harrison NL: $GABA_A$-R α 1 subunit knockin mutation leads to abnormal EEG and anesthetic-induced seizure-like activity in mice. Brain Res 2006; 1078:60–70
13. Dutton RC, Maurer AJ, Sonner JM, Fanselow MS, Laster MJ, Eger EI II: The concentration of isoflurane required to suppress learning depends on the type of learning. ANESTHESIOLOGY 2001; 94:514–9
14. Eger EI II, Xing Y, Pearce R, Shafer S, Laster MJ, Zhang Y, Fanselow MS, Sonner JM: Isoflurane antagonizes the capacity of flurothyl or 1,2-dichlorohexafluorocyclobutane to impair fear conditioning to context and tone. Anesth Analg 2003; 96:1010–8
15. Sonner JM, Cascio M, Xing Y, Fanselow MS, Kralic JE, Morrow AL, Korpi ER, Hardy S, Sloat B, Eger EI II, Homanics GE: Alpha 1 subunit-containing GABA type A receptors in forebrain contribute to the effect of inhaled anesthetics on conditioned fear. Mol Pharmacol 2005; 68:61–8
16. Dutton RC, Maurer AJ, Sonner JM, Fanselow MS, Laster MJ, Eger EI II: Isoflurane causes anterograde but not retrograde amnesia for pavlovian fear conditioning. ANESTHESIOLOGY 2002; 96:1223–9
17. Sonner JM, Gong D, Li J, Eger EI II, Laster MJ: Mouse strain modestly influences minimum alveolar anesthetic concentration and convulsivity of inhaled compounds. Anesth Analg 1999; 89:1030–4
18. Homanics GE, Ferguson C, Quinlan JJ, Daggett J, Snyder K, Lagenaur C, Mi ZP, Wang XH, Grayson DR, Firestone LL: Gene knockout of the α6 subunit of the γ-aminobutyric acid type A receptor: Lack of effect on responses to ethanol, pentobarbital, and general anesthetics. Mol Pharmacol 1997; 51:588–96
19. Quinlan JJ, Homanics GE, Firestone LL: Anesthesia sensitivity in mice that lack the β3 subunit of the γ-aminobutyric acid type A receptor. ANESTHESIOLOGY 1998; 88:775–80
20. Alifimoff JK, Firestone LL, Miller KW: Anesthetic potencies of secondary alcohol enantiomers. ANESTHESIOLOGY 1987; 66:55–9
21. Vicini S, Ferguson C, Prybylowski K, Kralic JE, Morrow AL, Homanics GE: $GABA_A$ receptor α1 subunit deletion prevents developmental changes of inhibitory synaptic currents in cerebellar neurons. J Neurosci 2001; 21:3009–16
22. Browne SH, Kang J, Akk G, Chiang LW, Schulman H, Huguenard JR, Prince DA: Kinetic and pharmacological properties of $GABA_A$ receptors in single thalamic neurons and $GABA_A$ subunit expression. J Neurophysiol 2001; 86:2312–22
23. Rudolph U, Mohler H: Analysis of $GABA_A$ receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. Annu Rev Pharmacol Toxicol 2004; 44:475–98
24. Liachenko S, Tang P, Somogyi GT, Xu Y: Comparison of anaesthetic and non-anaesthetic effects on depolarization-evoked glutamate and GABA release from mouse cerebrocortical slices. Br J Pharmacol 1998; 123:1274–80
25. Sonner JM, Zhang Y, Stabernack C, Abaigar W, Xing Y, Laster MJ: $GABA_A$ receptor blockade antagonizes the immobilizing action of propofol but not ketamine or isoflurane in a dose-related manner. Anesth Analg 2003; 96:706–12
26. Werner DF, Blednov YA, Ariwodola OJ, Silberman Y, Logan E, Berry RB, Borghese CM, Matthews D, Weiner JL, Harrison NL, Harris RA, Homanics GE: Knockin mice with ethanol-insensitive α1-containing γ-aminobutyric acid type A receptors display selective alterations in behavioral responses to ethanol. J Pharmacol Exp Ther 2006; 319:219–27
27. Hanchar HJ, Dodson PD, Olsen RW, Otis TS, Wallner M: Alcohol-induced motor impairment caused by increased extrasynaptic $GABA_A$ receptor activity. Nat Neurosci 2005; 8:339–45
28. Bieda MC, MacIver MB: Major role for tonic $GABA_A$ conductances in anesthetic suppression of intrinsic neuronal excitability. J Neurophysiol 2004; 92:1658–67
29. Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ: Extrasynaptic $GABA_A$ receptors of thalamocortical neurons: A molecular target for hypnotics. J Neurosci 2005; 25:11513–20
30. 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