Sevoflurane Blocks the Induction of Long-term Potentiation When Present during, but Not When Present Only before, the High-frequency Stimulation

Jinyang Liu, M.S., Lie Yang, M.D., Ph.D., Daisy Lin, Ph.D., James E. Cottrell, M.D., Ira S. Kass, Ph.D.

ABSTRACT

Background: This study tests the hypothesis that sevoflurane blocks long-term potentiation only if it is present during the high-frequency stimulation that induces long-term potentiation.

Methods: Long-term potentiation, an electrophysiologic correlate of memory, was induced by high-frequency stimulation and measured as a persistent increase in the field excitatory postsynaptic potential slope in the CA1 region.

Results: Long-term potentiation was induced in the no sevoflurane group (171 ± 58% *vs.* 96 ± 11%; n = 13, mean ± SD); when sevoflurane (4%) was present during the high-frequency stimulation, long-term potentiation was blocked (92 ± 22% *vs.* 99 ± 7%, n = 6). While sevoflurane reduced the size of the field excitatory postsynaptic potential to single test stimuli by 59 ± 17%, it did not significantly reduce the size of the field excitatory postsynaptic potentials during the 100 Hz high-frequency stimulation. If sevoflurane was removed from the artificial cerebrospinal fluid superfusing the slices 10 min before the high-frequency stimulation, then long-term potentiation was induced (185 ± 48%, n = 7); this was not different from long-term potentiation in the no sevoflurane slices (171 ± 58). Sevoflurane before, but not during, Θ-burst stimulation, a physiologic stimulus, did not block the induction of long-term potentiation (151 ± 37% *vs.* 161 ± 34%, n = 7).

Conclusions: Sevoflurane blocks long-term potentiation formation if present during the high-frequency stimulation; this blockage of long-term potentiation does not persist if sevoflurane is discontinued before the high-frequency stimulation. These results may explain why short periods of insufficient sevoflurane anesthesia may lead to recall of painful or traumatic events during surgery.
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ANESTHETICS, such as sevoflurane, were developed to allow patients a rapid reversibility to consciousness after the completion of surgical procedures.[1] This results in rapid postsurgical assessment of neurologic function, reduced time in the postanesthesia care unit, rapid patient turnover in the operating room, and lower cost. However, it is not clear whether this rapid reawakening is coincident with a rapid reversibility of memory formation. In this study, we examine sevoflurane for its effect on long-term potentiation (LTP), an electrophysiologic correlate of memory.[2–5] Some studies have found that volatile anesthetics block LTP formation, while other studies did not find a reduction in LTP with volatile anesthetics.[6–9] Specifically, we address the time during which sevoflurane application is necessary to block LTP and whether discontinuation of sevoflurane allows rapid recovery of LTP induction and thereby new memory formation.

Sevoflurane induces an immediate enhancement of the γ-aminobutyric acid type A ($GABA_A$) receptor and potassium (K) channel mediated inhibition, and a reduction of *N*-methyl-d-aspartate (NMDA) receptor mediated excitation; these effects are rapidly reversible.[1] If this is the mechanism by which sevoflurane blocks memory formation, then sevoflurane's effect on LTP should be rapidly reversed once the application of sevoflurane is discontinued. However, sevoflurane also has long-lasting effects on neurons; it improves recovery after ischemia if animals are preconditioned with sevoflurane minutes or days before the ischemia.[10,11] This preconditioning activates protein kinase C–related signaling pathways that have also been implicated in memory formation.[5,11] Thus,

What We Already Know about This Topic

- Sevoflurane administration, in appropriate doses, produces amnesia in part by facilitating γ-aminobutyric acid–mediated inhibition and decreasing N-methyl-D-aspartate–mediated excitation. These effects are rapidly reversed upon cessation of sevoflurane administration.
- Sevoflurane also triggers changes in signal transduction systems, some of which play a central role in memory formation that persist for some time. Whether these changes in signal transduction impact memory function after discontinuation of sevoflurane is not clear.

What This Article Tells Us That Is New

- Sevoflurane administration during the stimulation that induces long-term potentiation also reduced long-term potentiation, a model for memory formation, in the hippocampus. Long-term potentiation was not blocked if sevoflurane was discontinued before the stimulus that induces long-term potentiation.
- The results suggest that sevoflurane can suppress memory formation only during its administration. The persistent effects on signal transduction do not prevent the recovery of memory formation.

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sevoflurane may alter LTP after its removal if it causes a prolonged action on these kinase signaling pathways, which have been shown to be important for both memory formation and preconditioning.[5,10,11] LTP is induced by trains of high-frequency stimulation; after these trains, the responses to single stimuli are increased and this increase or potentiation can be maintained for hours in brain slices and days or longer in intact animals.[2–5] The high-frequency stimulation can be thought of as analogous to painful external stimuli that are exerted on a patient during surgery. The current study focuses on the timing of sevoflurane application and its effect on LTP induction, comparing when it is present only shortly before the high-frequency stimulation to when it is present during the high-frequency stimulation. We found that sevoflurane does not have an effect on LTP induction after its removal even though it causes prolonged changes in protein kinase C–related signaling. Depending on the timing of sevoflurane application during a surgical procedure and the duration of its efficacy to inhibit LTP induction, short periods of insufficient concentrations of sevoflurane could result in surgery-related memory.

Materials and Methods

The experiments were approved by the Institutional Animal Care and Use Committee of the State University of New York, Downstate Medical Center (Brooklyn, New York). Male C57BL/6 mice were anesthetized with 2% isoflurane for 2 min in a Plexiglass chamber (Fine Science Tools, USA). Adequate anesthesia was confirmed by the loss of the righting reflex and the lack of any response to handling. The animal was then decapitated with a guillotine, and its brain was quickly removed and placed into chilled (2 to 4°C) artificial cerebrospinal fluid (aCSF) that was equilibrated with 95% O_2–5% CO_2. The composition of the aCSF was, in mmol/l NaCl, 126; KCl, 3; KH_2PO_4, 1.4; $NaHCO_3$, 26; $MgSO_4$, 1.3; $CaCl_2$, 1.4; glucose, 4; at pH, 7.4. The hippocampus was rapidly removed from the brain and sliced. Hippocampal slices of 400 μm thickness were sectioned using a microtome advance manual tissue chopper. The slices were incubated in a beaker containing aCSF saturated with 95% O_2 and 5% CO_2, for 2 h at room temperature (approximately 25°C). Slices were transferred to a recording chamber and maintained at 37°C in this chamber during the electrophysiologic recording.[12,13]

Electrophysiologic Recording

Hippocampal slices were submerged in the physiologic recording chamber (Fine Science Tools) and perfused with aCSF at a rate of 3.0 ml/min. A bipolar stimulating electrode was placed in the Schaffer collateral pathway and the field excitatory postsynaptic potential (fEPSP) from the CA1 stratum radiatum was recorded with a glass-micropipette filled with 1 M NaCl. We measured the initial slope of the fEPSP to quantitate the postsynaptic responses. Signals were recorded using an Axoclamp 2B amplifier (Axon Instruments, USA), digitized, sampled at 20 KHz, and analyzed using pClamp 9 (Axon Instruments). The slices were stimulated with a constant current monophasic 0.1 ms pulse with intensity ranging from 0.2 to 1.0 mA.

Experiments were carried out at physiologic temperature (37°C) and glucose (4 mM) conditions. In the physiologic recording chamber, the slices were submerged 1 mm below the surface, the aCSF superfusing the slices was aerated with 95% O_2/5% CO_2 and this gas mixture was maintained in the atmosphere above the slices. We used the same technique and chamber to regularly record intracellularly from hippocampal neurons for over an hour; the neurons in these slices maintained normal excitability and resting potentials.[11,13,14] When sevoflurane was applied it was added to this gas mixture. We used a relatively high concentration of sevoflurane (4%); that is approximately twice the minimal alveolar concentration (2MAC). While we did not measure anesthetic concentration directly, we used a calibrated sevoflurane vaporizer (Penlon Sigma Elite, United Kingdom) to deliver sevoflurane in the gas stream to both the aCSF and the atmosphere above the slice. The top of the physiologic chamber was covered except for holes for viewing and to allow the electrodes into the slice; the gas flow was rapid and maintained the gas concentrations in the atmosphere above the slice. Indeed, previous experiments using this technique for sevoflurane-induced preconditioning found similar effects of the same concentration of sevoflurane in a study that compared slice and *in vivo* preparations.[15]

We used a stimulus that generated 30% of the maximum response as our test stimulus throughout the experiment; the slope of this response was plotted as 100%. Hippocampal slices from 6- to 12-week-old mice were either untreated (no sevoflurane) or treated with 4% sevoflurane at defined times during the experiment. A high-frequency stimulation protocol (two 1 s trains at 100 Hz separated by 20 s) was given to induce LTP. The no sevoflurane group received 50 min of superfusion without sevoflurane, followed by the high-frequency stimulation. The amplitude of the high-frequency stimulation was a stimulus that elicited 40% of the maximum response to stimulation. The slices were then superfused for an additional 60 min (n = 13). In addition to the test stimulus given every 30 s, a range of stimulus amplitudes were used to produce an input-output curve at two time points before and after the high-frequency stimulation. The sevoflurane-treated groups were first superfused with aCSF aerated without sevoflurane for 30 min, followed by 20 min aeration with 4% sevoflurane, and then 60 min without sevoflurane. For the sevoflurane during the high-frequency stimulation group, the high-frequency stimulation was given 15 min after the onset of sevoflurane application and sevoflurane was applied for an additional 5 min after the high-frequency stimulation (n = 6). In the sevoflurane before high-frequency stimulation group,

sevoflurane was given for 20 min and then removed from the gas mixture 10 min before the high-frequency stimulation was given (n = 7).

The next series of experiments examined hippocampal slices using different stimulus parameters and an aCSF that contained 10 mM glucose. The glucose concentration is higher than physiologic levels in the brain extracellular fluid; however, most other studies examining LTP in brain slices used 10 mM glucose. This higher level of glucose will allow us to compare our results directly to those of previous studies. In this group, the stimulus protocol for LTP induction was one that closely mimics stimulation that might occur during physiologic learning paradigms in animals.[16,17] This stimulus protocol consisted of four stimuli per burst at a 100 Hz frequency, each burst separated by 0.5 s, and there were 20 bursts per stimulus train (80 total stimuli per train). Two of these stimulus trains, separated by 20 s, were given to induce LTP. This stimulation protocol is called Θ-burst stimulation. While true Θ frequency burst stimulation would have a 0.2 s interval between bursts, we extended this time to 0.5 s to allow better recovery of the neurons between bursts. We did this because we were concerned that neurons in a slice are not as well perfused as neurons *in vivo* and might need longer to recover from each burst. The stimulation pattern we used, which has also been used by others examining brain slices, only mimics Θ-burst stimulation *in vivo*.[16,17]

Statistics

All the data are expressed as mean ± SD and were analyzed using Prism 4 (GraphPad Software, USA). All comparisons were specifically planned *a priori* to test three different scientific questions with respect to sevoflurane: (1) inhibition; (2) LTP at the end of the experiment; and (3) the stability of LTP during the last 30 min of the experiment. The difference between two groups was considered significant if $P < 0.05$, two-tailed. Student's *t* tests were used to analyze the difference in the slope of the fEPSP between the sevoflurane and no sevoflurane groups at 15 min of sevoflurane (to examine the maximal inhibitory effect of sevoflurane) and at 60 min after the high-frequency stimulation (to determine the effect of sevoflurane on LTP at the end of the experiment). In addition, two-way repeated measures ANOVA tests were used to determine if there was a significance difference in the slope of the fEPSP during the period from 30 to 60 min after the high-frequency stimulation and if there was an effect of sevoflurane on the fEPSP response during the high-frequency stimulation. We examine LTP in the period from 30 to 60 min after the high-frequency stimulation in order to eliminate any short-term effects of the high-frequency stimulation on the fEPSP. There was no explicit randomization of the animals; however, the mice are indistinguishable from each other precluding selection bias. The number of animals per group was determined from previous experience on the size and variability of the LTP effect independent of anesthetic effects.

Results

Effect of Sevoflurane during High-frequency Stimulation on LTP Induction

The effect of sevoflurane on memory formation was examined by applying it during the high-frequency stimulation and measuring its effects on LTP. The dendritic region CA1 response to a Schaffer collateral test stimulus that yields 30% of a maximal fEPSP response is shown in figure 1A, trace1, and figure 1B, trace 1. After high-frequency stimulation, the size of the response to the test stimulus is increased dramatically; this is shown in figure 1A, trace 2, 45 min after the high-frequency stimulation. A significant increase in the initial slope of the fEPSP is a commonly used and accepted measure of potentiation; the change in the mean slope throughout the experiment is shown in figure 1C. The slope of the fEPSP in the no sevoflurane slices significantly increased 1 h after the high-frequency stimulation compared to the slope of the fEPSP 30 min before high-frequency stimulation (171 ± 58% *vs.* 96 ± 11%; $P < 0.001$, n =13).

If sevoflurane was present during the high-frequency stimulation, the fEPSP response was not increased 45 min after the high-frequency stimulation (fig. 1B, trace 1 *vs.* fig. 1B, trace 2). The mean slope of the fEPSP in the CA1 region 60 min after the high-frequency stimulation did not increase compared to the slope of the fEPSP 30 min before high-frequency stimulation (92 ± 22% *vs.* 99 ± 7%, n = 6), thus sevoflurane during the high-frequency stimulation blocked LTP induction (fig. 1C). A two-way repeated measures ANOVA demonstrated a significant treatment effect ($P < 0.01$), analyzing the time points between 30 and 60 min after the high-frequency stimulation and comparing the no sevoflurane and the sevoflurane groups. There was no significant effect of time ($P = 0.44$), nor interaction between time and treatment ($P = 0.83$).

The data shown in figure 1C used a single test stimulus intensity that was set to yield a fEPSP slope, which was 30% of the maximal slope that could be obtained from a stimulus that gave the maximal response. In order to determine if weaker or stronger test stimuli also exhibited LTP, we did a stimulus response curve before and after the high-frequency stimulation for each slice. The increase in the slope of the fEPSP after the high-frequency stimulation was not limited to one stimulus intensity, there was an increased responsiveness after the high-frequency stimulation for all the stimulus strengths tested in the no sevoflurane group. Typical input-output response curves are shown for a slice in the no sevoflurane group before and after high-frequency stimulation (fig. 2A). We found a similar result for all animals/slices in the group. Sevoflurane, when present during the high-frequency stimulation, prevented the increase of the fEPSP at all stimulus intensities when measured 30 and 60 min after the high-frequency stimulation. This indicates the blockage of LTP

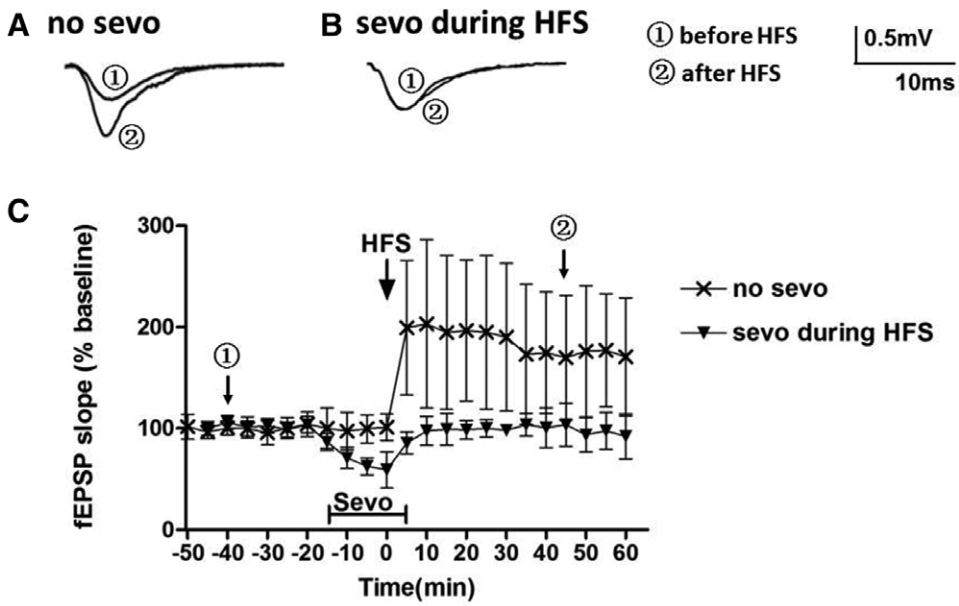

Fig. 1. The effect of sevoflurane during the high-frequency stimulation (HFS) on the induction of long-term potentiation (LTP). (*A*) Field excitatory postsynaptic potential (fEPSP) before (1) and after (2) the HFS in no sevoflurane slices; see (*C*) for the timing of these recordings. (*B*) fEPSP responses when sevoflurane was applied during the HFS: (1) before sevoflurane and HFS and (2) after sevoflurane and HFS. Application of sevoflurane during the HFS blocks the increase in the fEPSP 45 min later. (*C*) Sevoflurane reduces the fEPSP during its application. If sevoflurane is present during the HFS, the initial slope of the fEPSP does not increase above baseline after HFS; LTP induction is blocked by sevoflurane during the HFS. All values are the mean ± SD (n = 13, no sevoflurane group; n = 6, sevoflurane group).

induction; typical input-output curves are shown in figure 2B. Thus, at all stimulus levels examined, LTP was blocked when sevoflurane was present during the high-frequency stimulation.

Effect of Sevoflurane during High-frequency Stimulation on fEPSP during High-frequency Stimulation

In order to determine if sevoflurane was blocking LTP by reducing the fEPSP response to stimulation during the high-frequency stimulation, we examined its effect on the responses during the high-frequency stimulation used to induce LTP. Sevoflurane application (fig. 1C, −15 to +5 min) decreased the slope of the fEPSP (59 ± 17%) in the period before the high-frequency stimulation (fig. 1C). However, during the high-frequency stimulation there was no effect of sevoflurane on tetanic potentiation (fig. 3); the percentage was calculated by normalizing the fEPSP responses to the first stimulus in the high-frequency train. The fEPSP responded with robust responses during the high-frequency stimulation, even in the presence of sevoflurane; there was tetanic potentiation between the first and tenth stimulation in the train, and there was tetanic depression by the fiftieth stimulus of the train. The percentage change in fEPSP slope during the high-frequency stimulation was not significantly different with sevoflurane. The data were analyzed with a two-way repeated measures ANOVA; there was no treatment effect of sevoflurane (P = 0.16), there was an effect of stimulus number in the train (P < 0.001), and there was no interaction between stimulus number and treatment (P = 0.26).

Effect of Sevoflurane on LTP Induction if Discontinued before High-frequency Stimulation

In order to determine whether sevoflurane has a rapidly reversible or prolonged blocking effect on LTP, we carried out experiments that discontinued sevoflurane application 10 min before the high-frequency stimulation. The fEPSP response to a test stimulus 45 min after high-frequency stimulation is shown in figure 4A, trace 2; figure 4A, trace 1 is before sevoflurane and the high-frequency stimulation. The LTP was not attenuated if sevoflurane was applied and then discontinued before the high-frequency stimulation (fig. 4B, trace 2 *vs.* trace 1). This result was different from that obtained when sevoflurane was present during the high-frequency stimulation (fig. 1B, trace 2). The initial slope of the fEPSP, measured 60 min after the high-frequency stimulation in the sevoflurane before group, demonstrated robust LTP (185 ± 18; P < 0.001; n = 7; fig. 4). The fEPSP in the sevoflurane group was not significantly different from the no sevoflurane group at 60 min. There was no treatment effect if the time points between 30 and 60 min after the high-frequency stimulation were compared for the sevoflurane before high-frequency stimulation *versus* the no sevoflurane group using a two-way repeated measures ANOVA (P = 0.55; fig. 4); there was also no effect of time (P = 0.38) or interaction (P = 0.99).

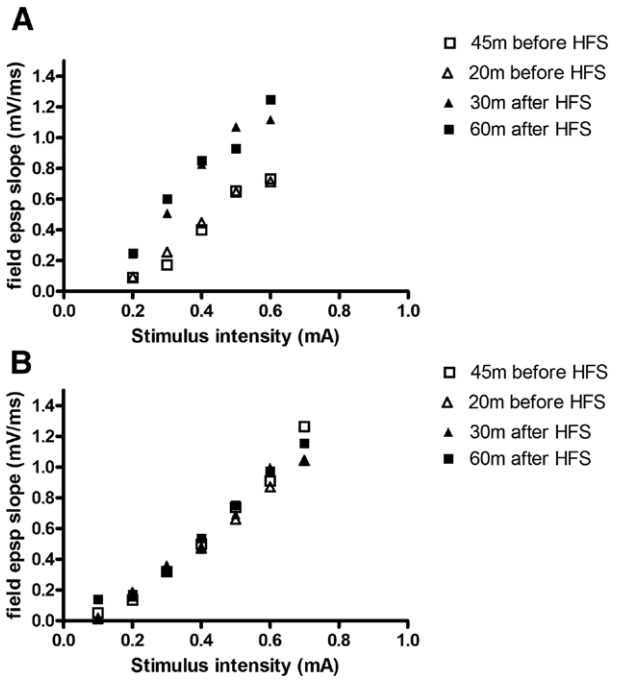

Fig. 2. The effect of sevoflurane during the high-frequency stimulation (HFS) on stimulus-response curves before and after the HFS. Typical stimulus-response plots are shown for individual experiments. The slope of the field excitatory postsynaptic potential (fEPSP) is plotted at a range of different stimulus intensities at four time points: 45 min before the HFS; 20 min before the HFS; 30 min after the HFS; 60 min after the HFS. (*A*) In a no sevoflurane slice, the fEPSP slopes are greater after the HFS throughout the range of stimulus intensities tested, and LTP was present at all stimulus intensities examined. (*B*) When sevoflurane was applied during HFS, no increase in the slopes of the fEPSPs were observed at any stimulus intensity; LTP was blocked at all stimulus intensities examined.

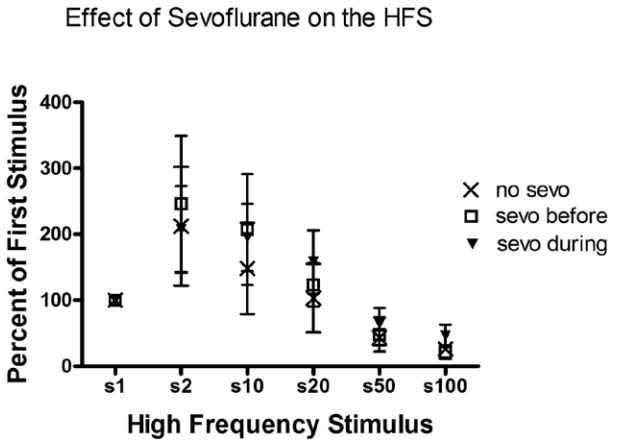

Fig. 3. The effects of sevoflurane on the field excitatory postsynaptic potential (fEPSP) response during the high-frequency stimulation. Values above 100% indicate tetanic potentiation; values less than 100% indicate tetanic depression. All values are normalized to the fEPSP of the first response in the high-frequency stimulation train of that group (mean ± SD). In all groups, there was significant tetanic potentiation from the second until the tenth stimulus and tetanic depression after the fiftieth stimulus. In the no sevoflurane group (n = 13), sevoflurane is not given at any time during the experiment. In the sevoflurane during group (n = 6), sevoflurane is present 15 min before and 5 min after the high-frequency stimulation. In the sevoflurane before high-frequency stimulation group (sevoflurane before), sevoflurane is present for 20 min and removed 10 min before the high-frequency stimulation (n = 7). There was no significant difference between the groups at any time point.

When the responses to different intensity stimuli were examined, the fEPSP increased for all stimulus intensities tested (fig. 5). Thus, sevoflurane, when discontinued 10 min before the high-frequency stimulation, did not block LTP induction.

Effect of Sevoflurane before, but Not during High-Frequency Stimulation on fEPSP during High-frequency Stimulation

Sevoflurane reduced the slope of the fEPSP during its application (50 ± 13%; $P < 0.001$); the response returned back to the no sevoflurane levels within 10 min of sevoflurane discontinuation (fig. 4C, time = 0 min). Sevoflurane given only before the high-frequency stimulation has no effect on the fEPSP during high-frequency stimulation (fig. 3). A two-way repeated measures ANOVA demonstrated no treatment ($P = 0.26$) or interaction effect ($P = 0.27$) but did find an effect of stimulus number in the train ($P < 0.001$). The effect of stimulus number in the train was similar in all groups: an initial increase due to tetanic potentiation followed by a decrease due to tetanic depression.

Effect of Sevoflurane on LTP Induction if Discontinued before Θ-burst Stimulation

In a different series of experiments, we examined whether sevoflurane has a rapidly reversible or prolonged blocking effect on LTP induction when the high-frequency stimulation parameters were a closer mimic of physiologic brain activity. This type of high-frequency stimulation, which is called Θ-burst, consists of multiple short bursts of stimuli instead of a long, continuous stimulus train. The intensity of a single test stimulus was adjusted to yield 30% of the maximal fEPSP response before Θ-burst stimulation; this response is shown in figure 6A, trace 1. A Θ-burst stimulation paradigm was then applied without any sevoflurane treatment and the fEPSP response 45 min after this high-frequency stimulation demonstrated an increase in response amplitude (fig. 6A, trace 2). There was a significant and sustained increase in the fEPSP slope after Θ-burst stimulation; it reached 161 ± 34% (n = 7) 60 min after the Θ-burst stimulation. This demonstrates successful LTP induction with Θ-burst stimulation. Sevoflurane was applied and then discontinued 10 min before Θ-burst stimulation. The response to a single test stimulation before sevoflurane and

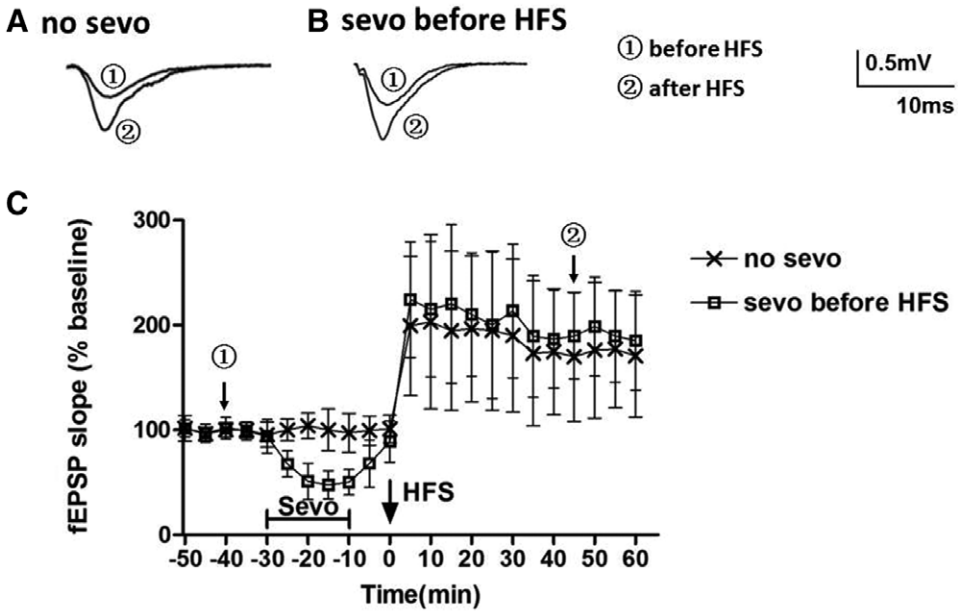

Fig. 4. The effect of sevoflurane before the high-frequency stimulation (HFS) on the induction of long-term potentiation (LTP). (*A*) Field excitatory postsynaptic potential (fEPSP) before (1) and after (2) the HFS in a slice not treated with sevoflurane (no sevoflurane); see (*C*) for the timing of these recordings. (*B*) fEPSP response from a slice treated with (sevoflurane) before the HFS: (1) before sevoflurane and HFS and (2) after sevoflurane and HFS. Sevoflurane before the HFS does not block the increase in the fEPSP after HFS. (*C*) Sevoflurane reduces the fEPSP during its application; the fEPSP returns to baseline after sevoflurane removal. If sevoflurane was applied and then discontinued before HFS, the initial slope of the fEPSP increases after the HFS. Sevoflurane discontinued before the HFS does not block LTP induction. All values are the mean ± SD (n = 7, sevoflurane before group; n = 13, no sevoflurane group).

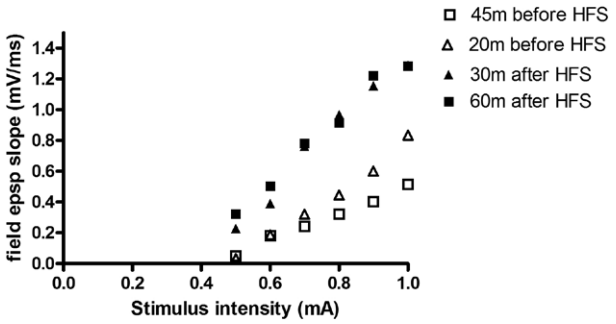

Fig. 5. The effect of sevoflurane withdrawal before high-frequency stimulation (HFS) on the stimulus-response curves before and after HFS. Typical stimulus-response plots are shown. The slope of the field excitatory postsynaptic potential (fEPSP) is plotted at a range of different stimulus intensities at four time points: 45 min before the HFS; 20 min before the HFS; 30 min after the HFS; and 60 min after the HFS. The slice had sevoflurane applied for 20 min and sevoflurane was removed 10 before the HFS (sevoflurane before). Throughout the entire range of stimulus intensities, the fEPSP slope is increased after the HFS; LTP was induced if sevoflurane application was discontinued before HFS.

Θ-burst stimulation (fig. 6B, trace 1) and after sevoflurane and Θ-burst stimulation (fig. 6B, trace 2) are shown; sevoflurane did not block LTP induction. We observed that the fEPSP increased to 151 ± 37% (n = 7) 60 min after Θ-burst stimulation in the sevoflurane before Θ-burst stimulation group. Sevoflurane did not reduce LTP when it was discontinued 10 min before the Θ-burst stimulation (fig. 6C). A two-way repeated measures ANOVA indicated there was no treatment effect if the time points between 30 and 60 min after the Θ-burst stimulation are compared for the sevoflurane before Θ-burst stimulation *versus* the no sevoflurane groups ($P = 0.79$); there were also no significant time ($P = 0.38$) or interaction ($P = 0.99$) effects. Thus, sevoflurane does not block LTP induction if it is removed 10 min before either continuous or Θ-burst high-frequency stimulation.

Effect of Sevoflurane on the fEPSPs during the Θ-burst Stimulation if Discontinued before Θ-burst Stimulation

Sevoflurane application significantly reduced the slope of the fEPSP in response to single stimuli (65 ± 11%); the response recovered 10 min after sevoflurane was discontinued (fig. 6C). Θ-Burst stimulation led to a continued strong response throughout the stimulus train (fig. 7); this was different from continuous 1 s stimulation, which led to tetanic depression after the fiftieth stimulus (fig. 3). A two-way repeated measures ANOVA of the fEPSP due to stimuli during the Θ-burst stimulation found no significant treatment effect when the no sevoflurane and sevoflurane groups were compared ($P = 0.17$); however, there was a significant burst ($P < 0.001$) and interaction ($P < 0.04$) effect.

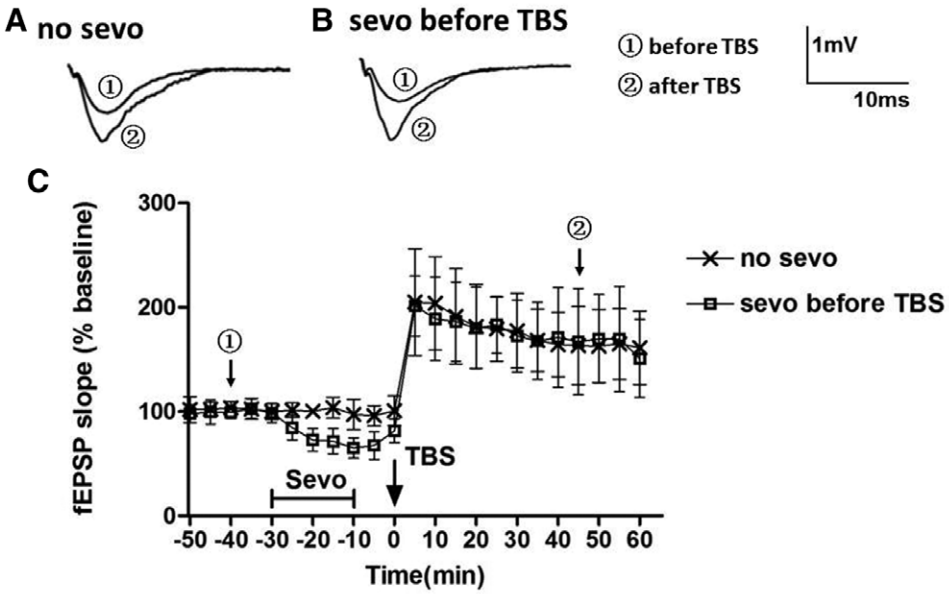

Fig. 6. The effect of sevoflurane before Θ-burst stimulation on the induction of long-term potentiation (LTP). (*A*) Field excitatory postsynaptic potential (fEPSP) before (1) and after (2) the Θ-burst stimulation (TBS) in slices not treated with sevoflurane (no sevoflurane); see (*C*) for the timing of these recordings. (*B*) fEPSP responses from slices treated with sevoflurane before TBS (sevoflurane before TBS): (1) before sevoflurane and TBS, and (2) after sevoflurane and TBS. (*C*) Sevoflurane reduces the fEPSP during its application. If sevoflurane application is discontinued before TBS, the initial slope of the fEPSP increases after TBS; LTP induction is not blocked by sevoflurane before TBS. All values are the mean ± SD; n = 7 for each group.

Discussion

Volatile anesthetics have been shown to impair learning and block memory formation when they are present at adequate concentrations.[1] However, if patients receive too little anesthetic for a short period of time, they can sometimes recall the events during surgery.[1,18] Rapid reversal, without a persistent effect to block learning and memory, could lead to the recall of events during surgery if anesthetic concentrations fall briefly. In this study, we investigated sevoflurane's ability to inhibit LTP, an electrophysiologic correlate of learning and memory, and whether sevoflurane's inhibition is rapidly reversed after its removal. We used mouse hippocampal slices since they allow rapid removal of the anesthetic from the tissue, quantification of electrophysiologic parameters, and, most importantly, the hippocampus has been shown to be important for spatial learning and memory.[19–21] In order to be certain that the anesthetic was rapidly removed minutes after the sevoflurane vaporizer was switched from 4 to 0%, we measured the direct effect of sevoflurane on inhibition of the fEPSP. The inhibitory effect of sevoflurane on the fEPSP slope was completely reversed 10 min after its removal from the gas mixture aerating the slices; this indicates rapid and effective anesthetic removal from the tissue.

In order to enhance their relevance, the experiments in this paper were done at 37°C; previous studies using the same techniques and chambers demonstrated stable intracellular responses at this temperature.[14] LTP is frequently studied at near physiologic temperatures (35°C) in brain slice preparations; however, anesthetic studies using brain slices have frequently been done at lower temperatures. Many anesthetic studies were done at 25°C.[6,7,9,22,23] Lower temperatures alter the efficacy and metabolic effects of anesthetics, which could lead to results that are not applicable to *in vivo* or clinical situations; therefore, we examined sevoflurane at physiologic temperatures, which is a unique aspect of our study.

Sevoflurane has immediate effects on ion channels associated with γ-aminobutyric acid and glutamatergic receptors, these effects are rapidly reversed when sevoflurane is removed; however, sevoflurane also affects kinase signaling pathways and these effects remain after the sevoflurane is removed.[1] Preconditioning of brain tissue with sevoflurane increased protein kinase M and protein kinase C expression hours and days after sevoflurane removal[15,24–26]; blocking the synthesis of these proteins blocks the anesthetic-induced improvement in recovery after ischemia.[11] Interestingly, protein kinase Mζ (PKMζ) has been shown to be critical for LTP maintenance, learning, and memory.[20,27,28] The preconditioning effects of anesthetics indicate they can lead to prolonged changes that remain after the anesthetic is discontinued.[11,24,29] Since sevoflurane blocked LTP induction when present during the high-frequency stimulation, but not if it was discontinued 10 min before the high-frequency stimulation, it is unlikely that sevoflurane's effects on kinase signaling pathways inhibit LTP formation. The action of sevoflurane to block LTP is likely upstream of the long-term changes of PKMζ activity or other cellular signaling pathways required for LTP induction and maintenance.

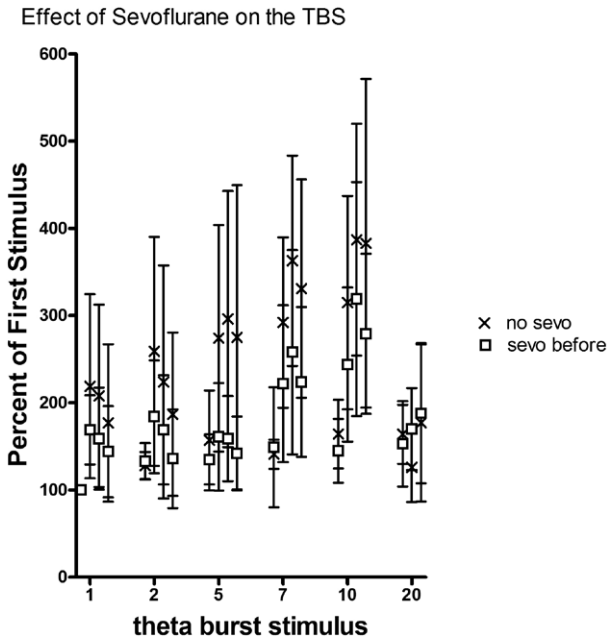

Fig. 7. The effect of sevoflurane before Θ-burst stimulation (TBS) on the field excitatory postsynaptic potential (fEPSP) during TBS. Each burst consists of four stimuli at a frequency of 100 Hz; there are 20 bursts per stimulus train; stimulus burst number is indicated on the abscissa. All values are normalized to the fEPSP in response to the first stimulus in the first burst (mean ± SD). Sevoflurane is present for 20 min and removed 10 min before TBS. A two-way ANOVA comparing the no sevoflurane and the sevoflurane before TBS groups found no significant treatment effect of sevoflurane on the fEPSP responses during TBS. TBS maintained a robust response throughout the stimulus period and showed no tetanic depression.

The stimulus-response data (figs. 2 and 5) show raw, unnormalized data for single slices, and the absolute stimulus intensities cannot be compared between slices due to differences in electrode placement and other variables. Comparisons before and after high-frequency stimulation in the same slice demonstrate that the changes in LTP are seen throughout the stimulus intensity range, not just at the test stimulus of 30% of maximum, and that the 30% of maximal stimulus intensity is in the range that would allow detection of an increase or decrease in LTP if it occurred.

We expected that after sevoflurane removal, its direct effects on ion channels no longer present, either an increase or decrease in LTP would occur because in previous experiments sevoflurane led to a sustained increased PKMζ, a kinase associated with LTP learning and memory. An increase in LTP could have been due to increased PKMζ in neurons enhancing synaptic efficacy; a decrease in LTP could have been due to the excess PKMζ occluding the ability of synapses to further increase the ability of synapses to increase efficacy. Since we detected no change over no sevoflurane LTP levels, this indicated sevoflurane had no effect on LTP after its washout. It is important and somewhat surprising that there are no sustained effects after sevoflurane removal even though it has a persistent effect on protein kinases C and M.[11,19,20]

We next examined whether the block of LTP induction by sevoflurane, when present during the high-frequency stimulation, could be explained by reduced excitation during the high-frequency stimulation. Sevoflurane reduced the fEPSP to a single stimulus during its application, but it had no significant effect on the fEPSP responses to the multiple stimuli during high-frequency stimulation. The stimulus intensity during the high-frequency stimulation was set to 40% of maximal at the beginning of the experiment for both the sevoflurane and no sevoflurane groups even though the fEPSP slope was less for first stimulus in the sevoflurane group. We wanted to examine the effects without adjustment because patients would not have increased excitability during sevoflurane, and it would have been impossible to adjust the responses during the train because train and single stimulation show different responses to sevoflurane; indeed, during the high-frequency stimulation there was no significant difference between groups. The fEPSP responses during the high-frequency stimulation were analyzed in detail; when normalized to the first response in the high-frequency stimulation train, there was no difference in tetanic potentiation during the high-frequency stimulation between the sevoflurane and no sevoflurane groups. This indicates that mechanisms of tetanic potentiation are not blocked by sevoflurane. Since sevoflurane did not significantly affect the responses to the high-frequency stimulation, it is likely working downstream of excitatory transmission.

Although studies examining LTP frequently use 1-s-long high-frequency stimulation trains, it is unusual to find stimuli of this duration *in vivo* without clinical pathology (*e.g.*, epilepsy)—the brain normally fires short bursts of activity.[16,17,30] Therefore, we used a train of short separated bursts (Θ-burst stimulation), which closely matches physiologic activation patterns in the brain.[16,17] A unique aspect of Θ-burst stimulation, when compared to a 1-s-long train of continuous high-frequency stimulation, is that the response to Θ-burst stimulation did not exhibit tetanic depression at the end of the trains and the potentiated response to stimulation is maintained throughout the Θ-burst stimulation. This physiologic stimulus paradigm also induced LTP 10 min after sevoflurane washout. Thus, we conclude there is no persistent effect of sevoflurane after 10 min of washout.

Our results indicate that if sevoflurane application is discontinued shortly before high-frequency stimulation, the LTP generated is similar to that in brain slices that were never exposed to sevoflurane. This suggests that the efficacy of sevoflurane to inhibit new memory formation would rapidly diminish after its withdrawal. The implication of our study is that maintaining adequate sevoflurane dosage during surgery is critical; reductions in the sevoflurane concentration at any time during surgery might impose risks of memory formation of painful or traumatic events during surgery.

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Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. Kass: Department of Anesthesiology HSC 6, SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, New York 11203–2098. ira.kass@downstate.edu.

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