Anesthesiology
2000; 92:228–36


# *Excitatory Synaptic Transmission Mediated by NMDA Receptors Is More Sensitive to Isoflurane than Are Non-NMDA Receptor-mediated Responses*

*Koh-ichi Nishikawa, M.D., Ph.D.,* M. Bruce MacIver, M.Sc., Ph.D.†*

***Background:*** **Effects of volatile anesthetic agents on *N*-methyl-D-aspartate (NMDA) receptor–mediated excitatory synaptic transmission have not been well characterized. The authors compared effects produced by halothane and isoflurane on electrophysiologic properties of NMDA and non-NMDA receptor–mediated synaptic responses in slices from the rat hippocampus.**

***Methods:*** **Field excitatory postsynaptic potentials (fEPSPs) in the CA1 area were recorded with extracellular electrodes after electrical stimulation of Schaffer-collateral-commissural fiber inputs. NMDA or non-NMDA receptor–mediated fEPSPs were pharmacologically isolated using selective antagonists. Clinically relevant concentrations of halothane or isoflurane were applied to slices in an artificial cerebrospinal fluid perfusate. Paired pulse facilitation was used as a measure of presynaptic effects of the anesthetic agents.**

***Results:*** **Clinically relevant concentrations of halothane (1.2 vol% ≈ 0.35 mM) depressed fEPSP amplitudes mediated by NMDA receptors and non-NMDA receptors to a similar degree (mean ± SD: 63.3 ± 14.0% of control, n = 5; 60.2 ± 7.3% of control, n = 7, respectively). In contrast, isoflurane (1.4 vol% ≈ 0.50 mM) preferentially depressed fEPSP amplitudes mediated by NMDA receptors (44.0 ± 7.4% of control, n = 6, $P < 0.001$) compared with those for non-NMDA receptors (68.7 ± 5.4% of control, n = 6), indicating a selective, additional postsynaptic effect. Paired pulse facilitation of fEPSPs was increased significantly by both anesthetic agents from 1.37 ± 0.13 to 1.91 ± 0.25 (n = 5, $P < 0.05$ for halothane) and from 1.44 ± 0.04 to 1.64 ± 0.08 (n = 5, $P < 0.01$ for isoflurane), suggesting that presynaptic mechanisms are also involved in fEPSP depression produced by the anesthetic agents. Neither rise times nor decay times of fEPSPs were changed in the presence of the anesthetic agents.**

***Conclusions:*** **These results indicate that fEPSPs mediated by postsynaptic NMDA receptors are more sensitive to clinically relevant concentrations of isoflurane than are non-NMDA receptor–mediated responses, but this selective effect was not observed for halothane. Both agents also appeared to depress release of glutamate from nerve terminals *via* presynaptic actions. (Key words: Anesthesia; CA1; glutamate; inhalational; kainate; rat hippocampal.)**

EXCITATORY synaptic transmission mediated by glutamate receptors appears to play several important and diverse roles in the mammalian central nervous system.[1] Based on pharmacologic sensitivity, ion permeability, and channel kinetics, the inotropic glutamate receptors are broadly divided into two subgroups determined by their specific agonists: *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors (*i.e.*, α-amino-3-hydroxy-5-methyl-4-isoxazole propionate, AMPA, and kainate receptors). NMDA receptor–mediated excitatory synaptic transmission may play a particularly important role for general anesthetic–induced neural depression and loss of recall[2]; however, effects produced by volatile anesthetic agents on NMDA and non-NMDA receptor–mediated synaptic responses have not been well characterized.

Previous studies have shown that volatile anesthetic agents can depress NMDA and non-NMDA receptor–mediated excitatory postsynaptic potentials (EPSPs).[3–5] For example, Perouansky *et al.*[6] reported that halothane depressed glutamatergic excitatory synaptic transmission irrespective of receptor subtype, most likely by inhibition of presynaptic release of glutamate. They also have reported that halothane similarly depressed the amplitudes of NMDA and non-NMDA receptor–mediated excitatory postsynaptic currents in hippocampal pyramidal cells[7] and inhibitory interneurons.[8] In contrast, it also has been reported that halothane may selectively block NMDA receptor channels. Population spikes mediated by NMDA receptors in the CA1 region of the rat hip-

* Postdoctoral Fellow.

† Associate Professor.

Received from the Department of Anesthesia, Stanford University School of Medicine, Stanford, California. Submitted for publication April 26, 1999. Accepted for publication August 4, 1999. Supported in part by grant no. GM54767 from the National Institutes of Health, Bethesda, Maryland; and by Professor Fumio Goto, Department of Anesthesiology, Gunma University School of Medicine, Maebashi, Japan (Dr. Nishikawa).

Address reprint requests to Dr. Nishikawa: Room S288, Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305-5117. Address electronic mail to: nishikaw@news.sb.gunma-u.ac.jp

pocampus appeared to be more sensitive to halothane than non-NMDA receptor–mediated responses.[9] In addition, biochemical studies also indicated that NMDA-stimulated uptake of $^{45}Ca^{2+}$ by a microvesicle fraction of the rat brain was sensitive to halothane (0.2–0.3 mM).[10] Thus, the relative degree of depression produced by volatile anesthetic agents on NMDA and non-NMDA receptor–mediated responses remains controversial, nor is it clear whether presynaptic or postsynaptic sites are involved for these anesthetic actions on excitatory synaptic transmission.

In this study, we investigated the effects of the volatile anesthetic agents halothane and isoflurane on NMDA and non-NMDA receptor–mediated field EPSPs (fEPSPs) in rat hippocampal slices and compared the effects of these agents on electrophysiologic properties of synaptic transmission.

## Methods

### *Brain Slice Preparation*

All electrophysiologic experiments were performed on brain slices isolated from young male Sprague-Dawley rats (weight, 55–70 g). Protocols were approved by the Institutional Animal Care Committee at Stanford University, and they adhered to published guidelines of the National Institutes of Health, Society for Neuroscience, and the American Physiologic Society. Rats were anesthetized with diethyl ether and killed by decapitation; brains were quickly removed and placed into cold (1–2°C), oxygenated artificial cerebrospinal fluids (ACSF). Brains were sectioned in the coronal plane into 500-μm-thick slices using a vibratome (Vibratome Series 1,000, Boston, MA). Slices were then hemisected and placed on filter papers at the interface of a humidified carbogen (95% $O_2$/5% $CO_2$) gas phase and ACSF liquid phase. Slices were allowed $\geq$ 1 h for recovery before submersion in ACSF in a recording chamber.

### *Electrophysiologic Recordings*

Field excitatory postsynaptic potentials were recorded in slices submerged in oxygenated ACSF as described previously.[5,11] The slices were constantly perfused with room-temperature (21–24°C) ACSF at a rate of 2.5 ml/min. fEPSPs were evoked using bipolar tungsten stimulating electrodes (Frederick Haer & Co., Bowdoinham, ME) placed in stratum radiatum of the CA1 regions to activate Schaffer-collateral-commissural (SCC) fibers (figs. 1A and 1B). Electrical stimuli consisted of square wave paired pulses from digitally timed and controlled isolation units (SUI5; Grass Instruments, Quincy, MA; 0.35 ms in duration, 60 ms interpulse interval, 4–8 V) were delivered every 15 s to minimize frequency-dependent changes in synaptic transmission. All fEPSPs were recorded using a glass microelectrode filled with ACSF (1–2 MΩ; Garner Glass Company, Claremont, CA). Recording electrodes were placed directly in line with stimulating electrodes in striatum radiatum but separated from stimulating electrodes by 1.0–1.5 mm. To block NMDA and non-NMDA receptor–mediated components, glutamate receptor antagonists (±)-2-amino-5-phosphonopentanoic acid (AP-5; 100 μM) or 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX; 17.2 μM) were added to the ACSF, respectively. In recordings of NMDA receptor–mediated synaptic responses, low $Mg^{2+}$ ACSF (0.2 mM) was used to facilitate an activation of NMDA receptor–gated $Ca^{2+}$ channels. In addition, stimulus intensity was increased (10 V) to recruit more presynaptic fibers leading to an increased release of glutamate. During these conditions, at least 1 h was required to achieve steady-state recordings of isolated NMDA receptor–mediated fEPSPs (see Results; fig. 1). Recording solutions also contained glycine (5 μM) to facilitate NMDA binding, and in some experiments bicuculline methiodide (10 μM) was added to block γaminobutyric acid (GABA)-mediated inhibitory responses. Signals were recorded with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA), amplified (at least 10,000 times), filtered at 10 kHz, and digitized at 50 kHz for computer storage and analysis (Data Wave Technologies, Longmont, CO). Response measures were plotted on line during experiments to ensure that stable baseline activity ($<$ 5% variation for 10 min) was achieved from each preparation before administration of the anesthetic agents. All experiments were performed at room temperature (21–24°C).

### *Application of Volatile Anesthetic Agents and Concentration Measurement*

Inhalation anesthetic agents were applied using a carrier gas (95% $O_2$/5% $CO_2$) and calibrated commercial vaporizers (Fluotec 3 for halothane, Fortec for isoflurane; Fraser Harlake, Orchard Park, NY). During experiments, the gas phase anesthetic concentrations in the ACSF reservoir were continuously monitored using a Datex 254 airway gas monitor (Datex, Helsinki, Finland). Aqueous phase concentrations of anesthetic agents were

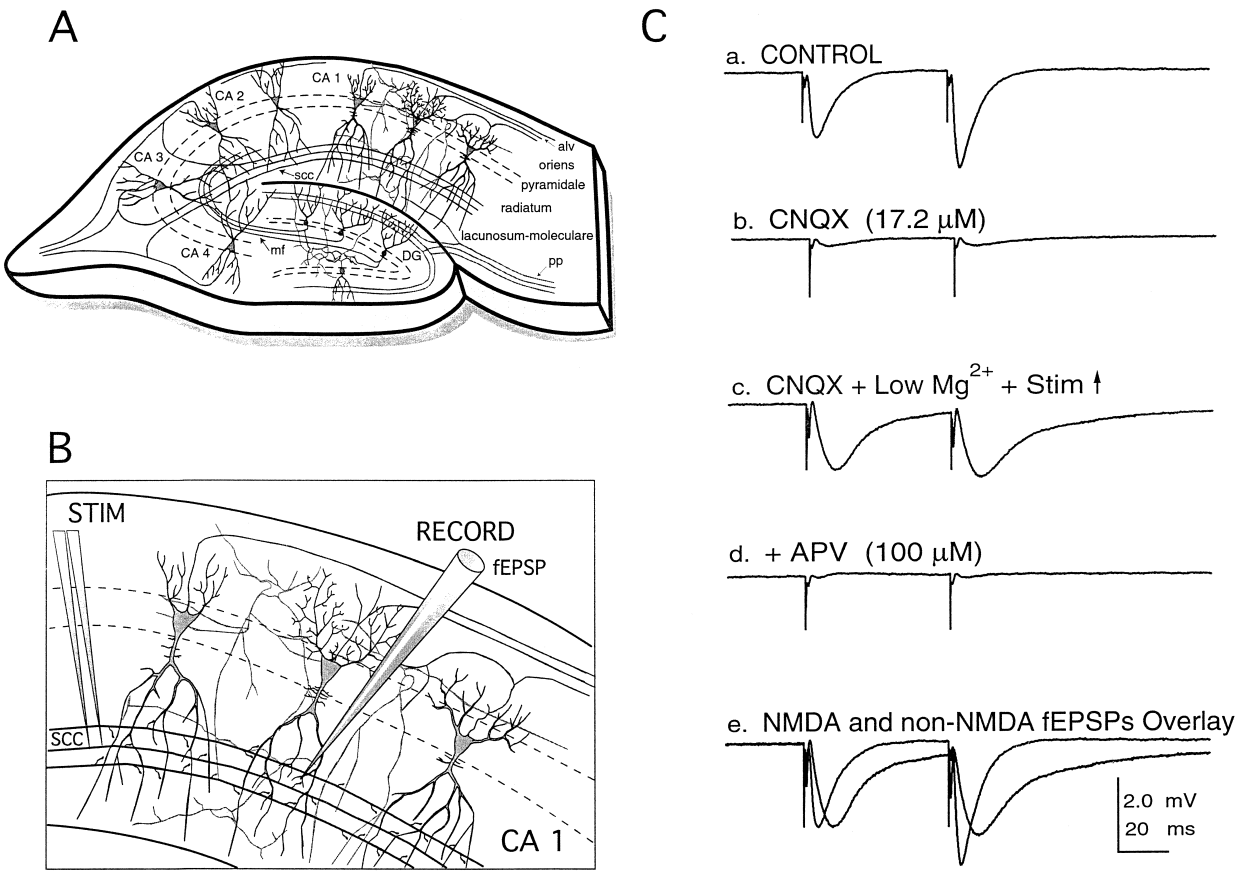


**Fig. 1. (*A*) Rat hippocampal slice formation showing the three major excitatory pathways of the hippocampus. The perforant pathway (pp) originates in the entorhinal cortex and projects to granule cells of the dentate gyrus (DG); the mossy fiber pathway (mf) is composed of granule cell axons and extends to the pyramidal cells of the area CA3; and the Schaffer-collateral-commissural (scc) pathway from CA3 provides glutamate-mediated excitatory synapses with dendrites of CA1 pyramidal cells and inhibitory interneurons. (*B*) Details of the area CA1 are shown; the placement of stimulating tungsten electrodes (STIM) and an extracellular recording electrode in striatum radiatum of the area CA1. SCC pathway axons, which are known to form excitatory synapses with dendrites of CA1 neurons, were stimulated electrically. (*C*) The fEPSPs mediated by glutamate receptors were evoked by a paired pulse stimulus to the SCC pathway. *a*, fEPSPs mediated by glutamate receptors were recorded in normal artificial cerebrospinal fluid (ACSF) solution. An interstimulus interval (60 ms) was used to obtain paired pulse facilitation. Note that facilitated responses to the second stimulus have a larger amplitude than first pulse responses. *b*, The AMPA/kainate receptor-mediated components were blocked with a competitive antagonist, CNQX (17.2 μM). Most of the glutamate receptor–mediated fEPSP was abolished, but CNQX-resistant components with small amplitudes could still be recorded. *c*, These CNQX-resistant components could be enhanced by increasing stimulus intensity and lowering the concentration of $Mg^{2+}$ (0.2 mM). *d*, Further addition of AP-5 (100 μM), an NMDA receptor antagonist, completely abolished fEPSPs, leaving the presynaptic fiber volley unaffected, indicating that these were NMDA receptor–mediated fEPSPs. *e*, An overlay of NMDA and non-NMDA receptor–mediated fEPSPs show the relatively slow time course of NMDA-fEPSPs.**

determined as reported previously.[12] Clinically relevant concentrations of volatile anesthetic agents (1.2 vol% ≈ 0.35 mM, 1 rat minimum alveolar concentration for halothane; 1.4 vol% ≈ 0.50 mM, 1 rat minimum alveolar concentration for isoflurane) were used throughout this study. Solutions containing anesthetic agents were changed rapidly and accurately using a computerized perfusion system (ValveBank8; AutoMate Scientific, Oakland, CA). High-quality polytetrafluorethylene was used for reservoirs, valves, and tubing to minimize loss of volatile anesthetic and drug binding.

*Data Analysis*

All responses were measured using DataWave software and analyzed using IGOR Pro software (WaveMetrics, Lake Oswego, OR). The amplitude of fEPSPs was measured from peak positive to peak negative voltages. Fiber volley amplitude was measured from baseline to peak

negative voltage. Rise times and decay times of fEPSPs were measured as the time interval between 10% and 90% of peak amplitude and as the decay time from peak amplitude to half amplitude, respectively. All responses were normalized as a percent of control, based on the average amplitude during a 10-min recording immediately before perfusion of the anesthetic agents. The statistical significance of data from two groups was determined using Student *t* tests. One-way analysis of variance (ANOVA) was used to compare the differences among three or more groups and to determine anesthetic effects with time-matched control responses.

*Materials and Chemicals*

All rats were obtained from Simonsen Laboratories (Gilroy, CA). Chemicals for the ACSF were regent grade or better and obtained from J. T. Baker (Philadelphia, PA) and Sigma Chemical Co. (St. Louis, MO). The ACSF had the following ionic composition (in mM): $Na^+$ 151.25, $K^+$ 3.5, $Ca^{2+}$ 2.0, $Mg^{2+}$ 2.0, $Cl^-$ 131.5, $HCO_3^-$ 26.0, $SO_4^-$ 2.0, $H_2PO_4^-$ 1.25, and glucose 10.0; *p*H was adjusted to 7.4.

## Results

*Isolation of fEPSPs Mediated by NMDA Receptors and Non-NMDA Receptors*

As shown in figure 1C, glutamate-mediated fEPSPs were recorded from CA1 neurons in stratum radiatum after paired pulse stimulation (60-ms intervals) of SCC fibers. Pharmacologic isolation of non-NMDA fEPSPs was obtained using a specific antagonist for NMDA receptors, AP-5 (100 $\mu$M). Consistent with a previous report,[13] we found that AP-5 had little effect on fEPSP amplitudes evoked by SCC pathway stimulation in normal ACSF solution (fig. 1Ca). NMDA-fEPSPs were isolated using an AMPA/kainate receptor blocker, CNQX (17.2 $\mu$M), after control fEPSPs were obtained in normal ACSF (fig. 1Cb). During these conditions, most of the glutamate receptor–mediated response was abolished. Residual CNQX-resistant components could be enhanced by lowering the external concentration of $Mg^{2+}$ (from 2.0 mM to 0.2 mM) and by increasing stimulus intensity (10 V; fig. 1Cc). At least 1 h was required to achieve full recovery of a synaptic response in the presence of CNQX. These responses remained stable for several hours and were completely abolished by application of the NMDA receptor antagonist AP-5 (100 $\mu$M; fig. 1Cd), indicating that they were NMDA receptor–mediated fEPSPs. An overlay of non-NMDA and NMDA receptor–mediated EPSPs clearly shows the relatively slow rise time (2.6 ± 0.4 ms and 5.7 ± 0.8 ms, n = 10, respectively) and prolonged decay time (12.3 ± 1.9 ms and 37.2 ± 7.1 ms, n = 12, respectively) of NMDA components compared with non-NMDA fEPSPs (fig. 1Ce).

*Volatile Anesthetic Agents Depressed Excitatory Synaptic Transmission*

Clinically relevant concentrations of halothane (1.2 vol% ≈ 0.35 mM) significantly depressed non-NMDA fEPSP and NMDA fEPSP amplitudes evoked by stimulation of the SCC pathway (mean ± SD, 60.2 ± 7.3% of control, n = 7, $P < 0.001$ *vs.* control; 63.3 ± 14.0% of control, n = 5, $P < 0.001$ *vs.* control, respectively; fig. 2). There was no significant difference between the degree of depression observed on both responses ($P = 0.68$). As shown in figure 3, isoflurane (1.4 vol% ≈ 0.50 mM) also had similar effects on non-NMDA fEPSP amplitudes (68.3 ± 5.8% of control, n = 5) with halothane ($P = 0.10$). Isoflurane, however, preferentially depressed NMDA fEPSP amplitudes (45.2 ± 7.5% of control, n = 5, $P < 0.001$ *vs.* control; fig. 3) compared with those for non-NMDA fEPSPs ($P < 0.001$).

Macroscopic onset and recovery kinetics of anesthetic effects were determined as the time constant ([$\tau$]) derived from fitting a single exponential function to the onset and the recovery phase of fEPSP amplitude depression. The onset time constant of halothane for NMDA fEPSPs (3.0 ± 1.3 min, n = 5) was significantly less than that for non-NMDA fEPSPs (6.6 ± 0.6 min, n = 7, $P < 0.001$). In contrast, no significant difference was observed for the recovery kinetics of halothane effects (6.6 ± 0.9 min for NMDA fEPSPs, n = 5 *vs.* 7.4 ± 1.1 min for non-NMDA fEPSPs, n = 7, $P = 0.18$). As seen with halothane, the onset time constant of isoflurane for NMDA fEPSPs (3.3 ± 1.3 min, n = 5) was significantly faster than that for non-NMDA fEPSPs (5.8 ± 0.8 min, n = 5, $P < 0.05$). In addition, the recovery time constant of isoflurane for NMDA fEPSPs (11.3 ± 1.6 min, n = 5) was significantly slower than that for non-NMDA fEPSPs (7.7 ± 1.5 min, n = 5, $P < 0.01$).

The faster time course for NMDA fEPSP depression suggests that these anesthetic agents might act on kinetically different sites at NMDA receptor–mediated synapses compared with non-NMDA fEPSPs. Alternatively, the faster kinetics could come about from an increased input/output coupling between glutamate and postsynaptic NMDA receptors. Anesthetic-induced depression of release of glutamate would then result in a faster

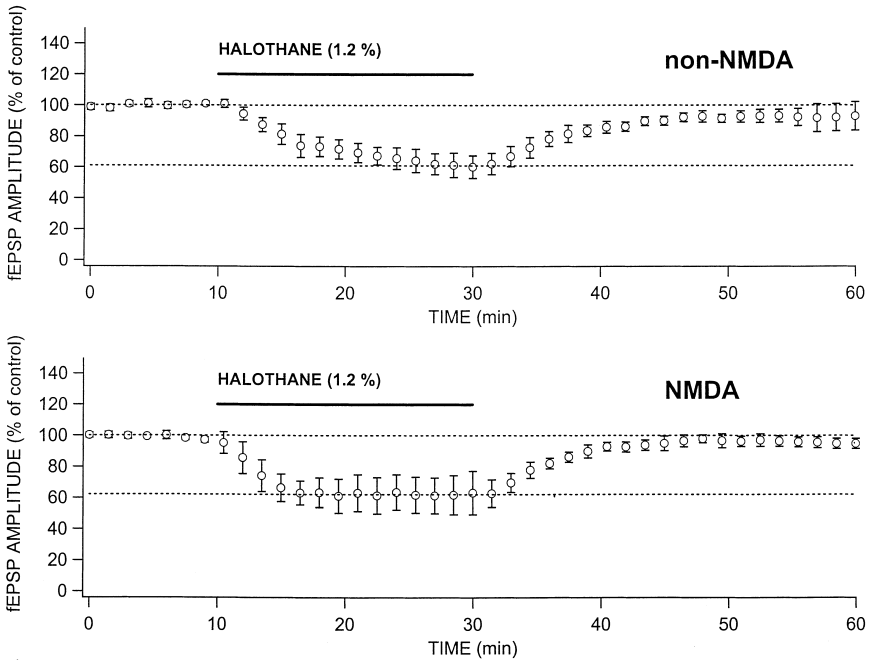


**Fig. 2. Halothane decreases fEPSP amplitudes mediated by non-NMDA receptors (*top*) and NMDA receptors (*bottom*) to a similar degree. Graphs of fEPSP amplitudes *vs.* experimental time show the halothane-induced depression of fEPSP amplitudes observed for non-NMDA and NMDA receptor–mediated responses. Each data point represents the mean ± SD from five experiments using separate slice preparations for NMDA responses and seven for non-NMDA responses. All amplitudes were expressed as a percent of average of control recordings taken during the 10 min preceding application of the anesthetic agent. Note that the time course of the effects of halothane on NMDA fEPSPs appeared to be faster than that for non-NMDA–mediated responses.**

apparent depression of NMDA receptor–mediated synapses. To examine this possibility, we evaluated the relation between fiber volley and fEPSP amplitude for NMDA and non-NMDA receptor–mediated responses at varying stimulus intensities. As shown in figure 4, a steeper relation was observed for NMDA fEPSPs, indicating that a small reduction in presynaptic release of glutamate leads to a larger change in fEPSP amplitude compared with non-NMDA responses. The effects of halothane and isoflurane on non-NMDA and NMDA fEPSP amplitudes are summarized in figure 5. Effects of isoflurane on NMDA fEPSPs were significantly greater

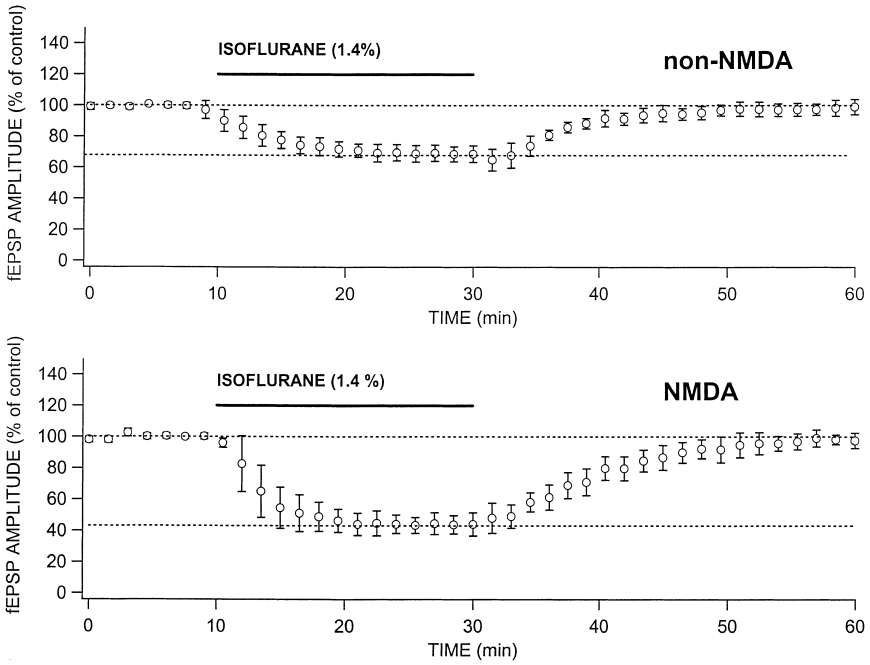


**Fig. 3. Isoflurane preferentially depressed the amplitude of fEPSPs mediated by NMDA receptors (*bottom*) compared with non-NMDA fEPSPs (*top*). This selective effect was significantly larger than that observed for non-NMDA fEPSPs ($P < 0.001$, ANOVA). Note that isoflurane has a faster onset and slower recovery phase of NMDA fEPSPs than those for non-NMDA fEPSPs. Each data point represents the mean ± SD from five experiments, each from different slices.**

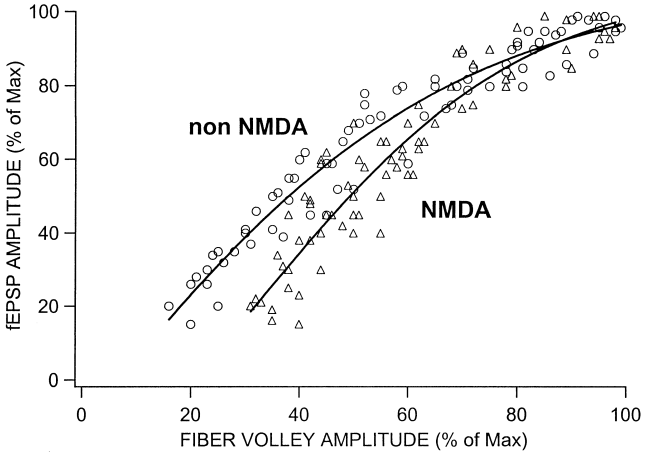


**Fig. 4. The relation of fEPSP amplitudes to fiber volley amplitudes with increasing stimulus intensities (4–10 V). Fit of fEPSP amplitudes *vs.* fiber volley amplitudes revealed that a steeper slope was observed for NMDA receptor–mediated responses. Both values were expressed as a percent of maximum amplitude. Data were obtained from three slices for non-NMDA fEPSPs (circles) and three slices for NMDA fEPSPs (triangles).**

than the effects of halothane or isoflurane on non-NMDA responses ($P < 0.001$, ANOVA).

### *Volatile Anesthetic Agents Increased Paired Pulse Facilitation of fEPSPs Mediated by Non-NMDA fEPSPs*

In recordings of paired pulse facilitation (PPF) of the SCC pathway, an interstimulus interval of 60 ms was used as described previously.[5] In control conditions using a stimulus intensity of ≈80% of maximal response, fEPSP facilitation was 1.35 ± 0.10 (n = 10) in normal ACSF. A 35% increase in amplitude of the second fEPSP was observed compared with the first (fig. 1C). Halothane and isoflurane increased facilitation of fEPSP amplitudes in normal ACSF (figs. 6A and 6B); however, these changes did not reach statistical significance with the small number of experiments (n = 5). It is likely that anesthetic-enhanced GABA-mediated feed forward inhibition also would contribute to this effect. To test this possibility, we studied the anesthetic-induced facilitation of fEPSPs in ACSF containing the $GABA_A$ receptor antagonist bicuculline (10 μM). Facilitation was slightly increased to 1.40 ± 0.11 (n = 10) in bicuculline-containing solutions, but no significant difference was observed ($P = 0.24$). Anesthetic effects on PPF were more clearly evident in data recorded in the presence of bicuculline (figs. 6C and 6D). During these conditions, halothane and isoflurane significantly increased facilitation of non-NMDA fEPSPs from 1.37 ± 0.13 to 1.91 ± 0.25 (n = 5, $P < 0.05$, ANOVA) and from 1.44 ± 0.04 to 1.64 ± 0.08 (n = 5, $P < 0.01$, ANOVA), respectively. These results indicate that anesthetic-enhanced GABA-mediated inhibition can have a significant effect on glutamate-mediated synaptic transmission.

### *Time Courses of fEPSPs Were Not Changed by Volatile Anesthetic Agents*

To examine the effects of volatile anesthetic agents on kinetic properties of postsynaptic glutamate receptors, effects on the time course of fEPSPs were analyzed. Six consecutive fEPSP recordings were averaged to reduce the effects of current fluctuations during individual fEPSPs. As shown in figure 1C, fEPSPs mediated by NMDA receptors had a relatively slow time course compared with non-NMDA fEPSPs. The rise time (time interval of 10–90% of peak amplitude) of non-NMDA fEPSPs was not changed by the anesthetic agents (2.6 ± 0.4 ms, n =

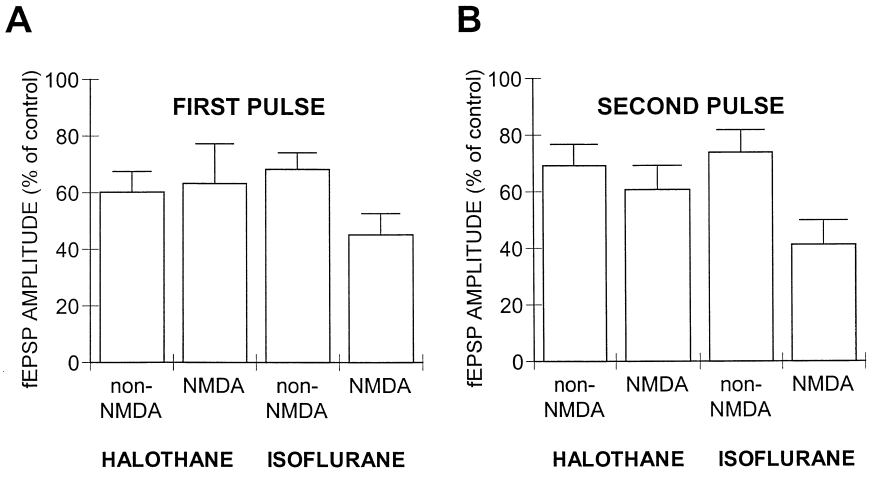


**Fig. 5. The effects of halothane and isoflurane on (*A*) first fEPSP amplitudes and (*B*) second fEPSP responses are summarized. An interstimulus interval (60 ms) was used to obtain paired pulse facilitation. The effects of isoflurane on NMDA fEPSPs were significantly greater than for those of halothane or isoflurane on non-NMDA responses (n = 7 for halothane on non-NMDA fEPSPs, n = 5 for the other three responses, mean ± SD, $P < 0.001$, ANOVA).**

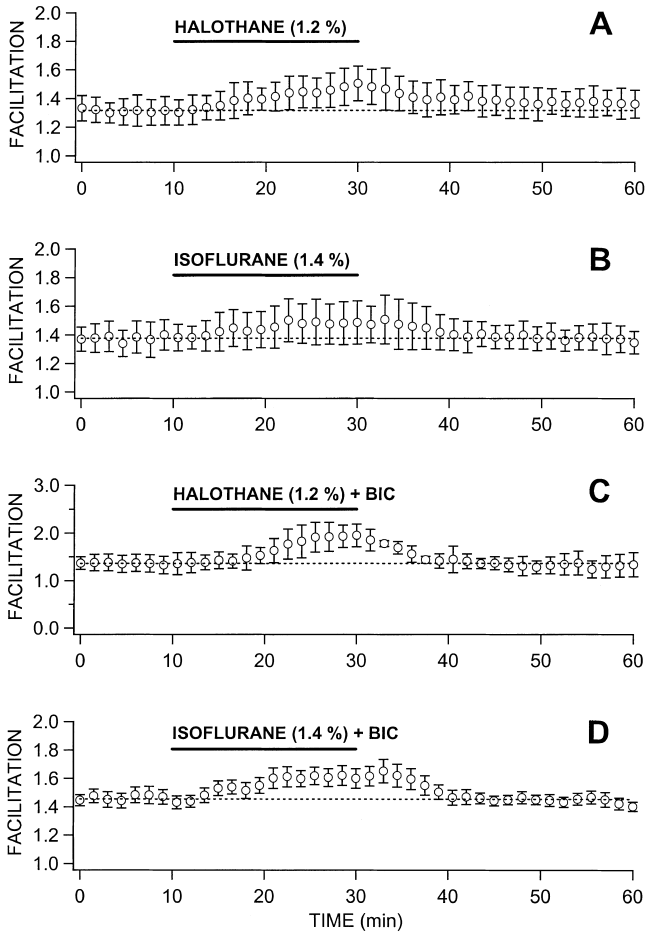


**Fig. 6. Paired pulse facilitation (PPF), measured as a ratio of second pulse amplitude divided by first pulse amplitude, was increased in the presence of the anesthetic agents. (*A*) PPF (pulse interval 60 ms) was recorded in the presence of halothane (1.2 vol% ≈ 0.35 mM) in normal artificial cerebrospinal fluid (ACSF). Halothane slightly increased PPF, but this effect was not significant compared with control (mean ± SD, n = 5, *P* = 0.16, ANOVA). (*B*) Similarly, isoflurane (1.4 vol% ≈ 0.50 mM) increased PPF studied in normal ASCF, but a significant difference was not observed with this small number of experiments (n = 5, *P* = 0.96, ANOVA). (*C*) PPF was recorded in ACSF containing bicuculline (10 μM). During this condition, halothane (1.2 vol% ≈ 0.35 mM) significantly increased PPF (mean ± SD, n = 5, *P* < 0.05 *vs.* control, ANOVA). (*D*) Isoflurane (1.4 vol% ≈ 0.50 mM) did significantly increase PPF in ACSF containing bicuculline (10 μM; n = 5, *P* < 0.01 *vs.* control, ANOVA), indicating that enhanced feed forward GABA-mediated inhibition can partially antagonized the anesthetic-induced increase in PPF.**

10 in control; 2.7 ± 0.3 ms, n = 5 in halothane; 2.8 ± 0.5 ms, n = 5 in isoflurane; *P* = 0.76). Similarly, no change in rise times was observed for NMDA fEPSPs in the presence of the anesthetic agents. In contrast, halothane had a prolonging tendency for the decay time (time interval from peak to half amplitude) of NMDA fEPSPs (from 37.8 ± 6.4 ms to 48.0 ± 13.2 ms, n = 6); however, this effect was not statistically significant (*P* = 0.12). These results indicate that the kinetic properties of the synaptic currents underlying fEPSPs were not appreciably changed by the anesthetic agents.

## Discussion

The major findings of the current study were that isoflurane preferentially depressed the amplitude of fEPSPs mediated by NMDA receptors, indicating that excitatory synaptic transmission mediated by NMDA receptors is more sensitive to isoflurane than are non-NMDA receptor–mediated responses, but this selective effect was not observed for halothane. In addition, our results confirm earlier findings that PPF of fEPSPs was significantly increased by both volatile anesthetic agents, suggesting that a main site of anesthetic action on glutamate-mediated synapses is presynaptic—to depress the release of glutamate from nerve terminals.[5,6,14]

### *Halothane Depressed NMDA and Non-NMDA Receptor–mediated fEPSPs*

Previous electrophysiologic studies indicated that clinically relevant concentrations of volatile anesthetic agents could inhibit glutamate receptor–mediated synaptic responses.[2–5,15] The relative degree of depression observed for NMDA and non-NMDA receptor–mediated synaptic responses remains controversial, however.[6,9] For example, halothane inhibited NMDA and non-MNDA receptor–mediated excitatory postsynaptic currents in pyramidal cells[7] and interneurons[8] of the hippocampal CA1 area. The 50% depression of NMDA excitatory postsynaptic currents amplitude produced by 0.57 mM halothane was similar to that seen in the current study. In contrast, it also has been reported that halothane appeared to selectively block NMDA receptor–mediated population spikes recorded from the rat CA1 area.[9] Our results agree with the former report that halothane appears to depress the amplitudes of NMDA and non-NMDA receptor–mediated fEPSPs to a similar degree. A previous study also has indicated that enflurane inhibited the NMDA-, AMPA-, and kainate-induced currents in oocytes expressing mouse mRNA by 29–40%, 30–33%, and 20–27%, respectively,[16] suggesting that all three glutamate inotropic receptors are susceptible to depression by volatile anesthetic agents. Our results provide further evidence that NMDA and non-NMDA receptor–mediated responses are equally sensitive to halothane.

*Isoflurane Selectively Depressed NMDA Receptor–mediated fEPSPs*

To our knowledge, the current study was the first to investigate the role of NMDA receptors in isoflurane actions on synaptic transmission of rat hippocampus. Excitatory synaptic transmission mediated by NMDA receptors appeared to be more sensitive to isoflurane than non-NMDA receptor–mediated responses. The reason for this selective difference of isoflurane on NMDA fEPSPs is unknown; however, we postulate additional postsynaptic actions. As mentioned earlier, halothane produced a similar degree of depression for NMDA and non-NMDA responses. In addition, it should be noted that isoflurane had equal effects on non-NMDA fEPSPs; however, isoflurane produced a greater than expected depression of NMDA responses (figs. 2, 3, and 5). The simplest explanation for the different effects produced by halothane and isoflurane would be that both anesthetic agents produce a presynaptic depression that accounts for most of the effects observed, consistent with the similar degree of PPF observed for both agents, and that isoflurane also acts postsynaptically to produce the extra depression seen only for isoflurane on NMDA fEPSPs. We suggest that selective effects of isoflurane on NMDA-mediated responses may involve postsynaptic receptor proteins and that NMDA receptors might be an important target for isoflurane.

Volatile anesthetic agents have been shown to decrease the mean open time of nicotinic acetylcholine receptor channels of embryonic *Xenopus* skeletal muscles in culture without affecting conductance.[17] Isoflurane may have a similar effect on NMDA receptor channels, although significant effects of isoflurane on decay times of fEPSPs were not observed in our experiments. Similar profiles of selective and differential effects of anesthetic agents on NMDA and non-NMDA receptors were also evident for other agents. NMDA receptor channels may be particularly sensitive to some intravenously administered anesthetic agents, for example, low concentrations of ketamine and phencyclidine inhibit NMDA but not AMPA/kainate-operated channels,[18,19] although pentobarbital showed an opposite selectivity, inhibiting kainate- but not NMDA-mediated responses.[20,21] This selective effect is similar to that seen with ethanol on NMDA receptor–mediated synaptic currents.[22,23] Thus, depression of NMDA receptor–mediated currents appears to play an important role for some anesthetic agents such as ketamine and alcohol, whereas the volatile agents appear to effect both types of glutamate synapses. It should be noted that effects on NMDA and non-NMDA receptor–mediated synapses occur at the same concentrations that increase GABA-mediated synaptic inhibition in this preparation. The depression of glutamate-mediated responses, however, occurs independently of the effects on GABA-mediated inhibition, because depression also was observed in the presence of the GABA receptor antagonist bicuculline.

*Paired Pulse Facilitation Was Increased by Volatile Anesthetic Agents*

Paired pulse facilitation of fEPSPs was used as a measure of presynaptic anesthetic actions at SCC fiber synapses on CA1 pyramidal neurons.[5,6,24] It has been shown that PPF increases after manipulations that reduce calcium-mediated release of glutamate from the SCC pathway.[24] In contrast, manipulations that depress CA1 neuron fEPSPs *via* postsynaptic actions do not change PPF.[24,25] The increased facilitation produced by halothane and isoflurane indicates that presynaptic sites are involved in depression of fEPSP amplitude. Recently, it has been shown that halothane presynaptically depressed glutamate-mediated synaptic transmission at the *Drosophila* neuromuscular junctions, but this effect was not observed for halothane-resistant mutants,[26] suggesting that a presynaptic action of halothane might account for not only synaptic depression but also some behavioral responses seen *in vivo.* A presynaptic action of halothane would account for the equi-effective depression of non-NMDA and NMDA receptor–mediated EPSPs and a lack of halothane effect seen for exogenously applied glutamate responses.[6,7]

Although we have focused on effects produced by volatile anesthetic agents on glutamate-mediated excitatory synaptic transmission, several electrophysiologic and biochemical studies have shown that the anesthetic agents enhance inhibitory synapses mediated by $GABA_A$ and glycine receptors. Pearce *et al.*[4] have reported that time courses of inhibitory synapses of CA1 neurons were greatly prolonged by volatile anesthetic agents. Enhancement of GABA-mediated chloride currents by volatile anesthetic agents was also reported in cultured rat hippocampal neurons.[27,28] These findings support the hypothesis that postsynaptic $GABA_A$ receptor channel complexes play an important role in general anesthesia (reviewed by Tanelian *et al.*[29]). Recently, more direct actions of volatile anesthetic agents on $GABA_A$ and glycine receptor proteins have been reported using point mutagenesis.[30] In our experiments, the anesthetic agents increased facilitation to a greater extent in the presence of bicuculline, suggesting that anesthetic-enhanced

GABA-mediated inhibition can have an important effect on glutamate-mediated synaptic transmission in slices.

Our results confirm earlier findings that clinically relevant concentrations of volatile anesthetic agents depress glutamate-mediated synaptic transmission *via* presynaptic mechanisms.[3,5–7,14,26] Isoflurane appeared to selectively inhibit NMDA receptor-mediated synaptic transmission, indicating additional postsynaptic actions for this agent. Taken together with the growing evidence for presynaptic and postsynaptic effects at GABA-mediated synapses, these results support a multisite agent-specific mechanism for anesthetic actions.[31–33]

The authors thank Frances A. Monroe for technical assistance and help illustrating the figures.

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