

Sevoflurane Blocks Cholinergic Synaptic Transmission Postsynaptically but Does Not Affect Short-term Potentiation

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Background: As compared with their effects on both inhibitory and excitatory synapses, little is known about the mechanisms by which general anesthetics affect synaptic plasticity that forms the basis for learning and memory at the cellular level. To test whether clinically relevant concentrations of sevoflurane affect short-term potentiation involving cholinergic synaptic transmission, the soma–soma synapses between identified, postsynaptic neurons were used.

Methods: Uniquely identifiable neurons visceral dorsal 4 (presynaptic) and left pedal dorsal 1 (postsynaptic) of the mollusk *Lymnaea stagnalis* were isolated from the intact ganglion and paired overnight in a soma–soma configuration. Simultaneous intracellular recordings coupled with fluorescent imaging of the FM1-43 dye were made in either the absence or the presence of sevoflurane.

Results: Cholinergic synapses, similar to those observed *in vivo*, developed between the neurons, and the synaptic transmission exhibited classic short-term, posttetanic potentiation. Action potential–induced (visceral dorsal 4), 1:1 excitatory postsynaptic potentials were reversibly and significantly suppressed by sevoflurane in a concentration-dependent manner. Fluorescent imaging with the dye FM1-43 revealed that sevoflurane did not affect presynaptic exocytosis or endocytosis; instead, postsynaptic nicotinic acetylcholine receptors were blocked in a concentration-dependent manner. To test the hypothesis that sevoflurane affects short-term potentiation, a posttetanic potentiation paradigm was used, and synaptic transmission was examined in either the presence or the absence of sevoflurane. Although 1.5% sevoflurane significantly reduced synaptic transmission between the paired cells, it did not affect the formation or retention of posttetanic potentiation at this synapse.

Conclusions: This study demonstrates that sevoflurane blocks cholinergic synaptic transmission postsynaptically but does not affect short-term synaptic plasticity at the visceral dorsal 4–left pedal dorsal 1 synapse.

THE inhalation anesthetics required during most surgical procedures affect both excitatory and inhibitory synaptic transmission in the nervous system.¹ These effects

involve either the suppression of presynaptic transmitter release² or a modulation of postsynaptic receptors.^{3,4} In contrast to their well-defined actions on both γ -aminobutyric acid–mediated⁵ and glutamatergic⁶ synapses, less understood are their effects on cholinergic transmission,^{7,8} which is thought to be involved in learning and memory in the hippocampus.

Inhalation and intravenous anesthetics are both thought to impair memory and exhibit potent amnesic properties. For example, patients who followed instructions intraoperatively did not recall such events on recovery.^{9,10} Similarly, other studies have demonstrated that both implicit and explicit memory states in humans are affected to some degree, by a varying state of anesthesia.¹¹ Reinsel *et al.*¹² have also demonstrated that memory is impaired during conscious sedation. From both psychologists¹³ and anesthesiologists¹⁴ points of view, memory for events during anesthesia has not been demonstrated.¹⁵ In contrast, numerous other studies have found no effect of anesthetics on various types of memories.¹⁶ For example, not only is the brain able to process auditory information during anesthesia,^{17–21} but also the cognitive functions required for memory remain unperturbed.²²

The above-cited examples provide ample reasoning to conclude that the issue whether anesthetics affect memory formation and retention at the cellular and network level remains polemical. This lack of fundamental knowledge in the field of anesthesiology *vis-à-vis* synaptic plasticity stems from the complex nature of the mammalian brain, where cell–cell interactions between well-defined sets of functionally identified, presynaptic and postsynaptic neurons can not be studied directly.

Here, we demonstrate that the clinically relevant concentrations of sevoflurane affect cholinergic synaptic transmission between well-defined synaptic partners and that these effects involve postsynaptic acetylcholine receptors. Moreover, we provide direct evidence that despite its effects on synaptic transmission, sevoflurane does not prevent posttetanic potentiation (PTP) at this synapse. Similarly, sevoflurane application after the induction of synaptic plasticity (potentiation paradigm: tetanus in the presynaptic cell) did not prevent the subsequent expression of PTP. Taken together, our data provide the first direct evidence that despite their effects on synaptic transmission, an inhalation anesthetic does

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not affect synaptic plasticity seen at an excitatory cholinergic synapse.

Materials and Methods

Animals

Laboratory-raised stocks of the fresh water snail *Lymnaea stagnalis* were maintained at room temperature (18°–20°C) in well-aerated aquaria and fed lettuces. Animals with shell lengths of 1–15 and 15–25 mm (approximate age, 2–6 months) were used for cell isolation and to prepare the brain conditioned medium, respectively. (Animal care certification is not required for invertebrate species such as *L. stagnalis* at the University of Calgary Animal Resource Centre, Calgary, Alberta, Canada).

Neuronal Culture

Identified neurons were isolated from the intact ganglia according to previously, well-established procedures in the laboratory.^{23,24} In summary, snails were anesthetized with 10% Listerine (Pfizer Canada, Toronto, Ontario, Canada) (21.9% ethanol, 0.042% menthol) solution in normal *Lymnaea* saline (containing 51.3 mM NaCl, 1–7 mM KCl, 4.1 mM CaCl₂, and 1.5 mM MgCl₂). HEPES was used to adjust the pH to 7.9. The central ring ganglia were dissected under sterile conditions as described previously²⁵ and washed in a series of antibiotic saline (50 µg/ml gentamycine; three washes, 10 min each). The antibiotic-treated ganglia were then incubated in 0.2% trypsin (Sigma type III; Sigma Chemical Company, St. Louis, MO) for 20–22 min followed by 0.2% soybean trypsin inhibitor (Sigma type 1-S; Sigma Chemical Company) for 10 min, both in defined medium (DM). DM consisted of serum-free 50% L-15 medium with added inorganic salts at a concentration described above for saline, and the pH was adjusted to 7.9 with 1 N NaOH. As compared with the antibiotic saline, the gentamycine concentration in DM was reduced to 20 µg/ml. The enzyme-pretreated ganglia were pinned down to the bottom of a dissection dish containing 6–10 ml high-osmolarity DM (DM + 37.5 mM glucose). The connective tissue sheath surrounding the ganglia was removed with fine forceps, and the desired neurons were isolated by applying gentle suction to a fire-polished and Sigmacote (Sigma Chemical Company)-treated pipette. The individually extracted cells were plated on poly-L-lysine-coated dishes containing brain conditioned medium, which was prepared by incubating central ring ganglia in DM (12 brains/6 ml DM for either 4 or 5 days). The isolated somata were juxtaposed in a soma-soma configuration.²⁶

Soma-Soma Synapse

Soma-soma synapses were prepared by juxtaposing the isolated somata of identified neurons as described previously.²⁶ Specifically, identified presynaptic neuron

(visceral dorsal 4 [VD4]) was isolated and paired with its postsynaptic partner (left pedal dorsal 1 [LPeD1]), and synapses were allowed to develop overnight in conditioned medium.

Electrophysiology

Well-established sharp electrode, intracellular recordings were made as described previously.²⁴ Briefly, glass microelectrodes (1.5-mm internal diameter W/Fil; World Precision Instruments, Sarasota, FL) were fabricated on a vertical electrode puller (Kopf, 700 C; David Kopf Instruments, Tujunga, CA) and filled with a saturated solution of K₂SO₄ (resistance 30–60 mΩ). Isolated neurons were viewed under a Zeiss (Telaval 31; Carl Zeiss Canada Ltd., North York, Ontario, Canada) inverted microscope and impaled using Narishigi micromanipulators (model MO-103; Narishigi Instruments, Tokyo Japan). The intracellular signals were amplified *via* a preamplifier (Neurodata model IR-283; Cygus Technology Inc., Delaware, PA), displayed on a storage oscilloscope (Tektronix R5103N; Tektronix, Montreal, Quebec, Canada), and recorded on a chart recorder (Gould; Gould Instrument Systems, Babylon, NY). All experiments were performed at room temperature (18°–22°C). The tetanus comprised 8–10 action potentials, and the posttetanic action potential was delivered after 6 s of the tetanus.

Anesthetic Delivery

Sevoflurane (Maruishi Pharmaceuticals Inc., Osaka, Japan) was vaporized in 100% oxygen using a sevoflurane type-S MKIII-VIII (Acoma Medical Industry Co., Tokyo, Japan) vaporizer and bubbled for at least 15 min into the reservoirs containing *Lymnaea* saline.²⁷ To minimize gas loss over time, all anesthetic solutions were prepared fresh in sealed glass reservoirs. Precise anesthetic concentrations were determined by gas chromatographic analysis established previously in our laboratory.²⁷ To minimize gas loss, Teflon tubing was used throughout the perfusion system, and sevoflurane was delivered directly to the somata using a fast perfusion system as described previously.²⁸ Acetylcholine (1 µM) was pressure applied (80-ms pulses, 2–4 psi) directly onto the somata *via* a pneumatic PicoPump (PV 800; World Precision Instruments).

FM1-43

The paired somata of presynaptic and postsynaptic neurons were incubated in 20 µM FM1-43 (Molecular Probes; Invitrogen Canada Inc., Burlington, Ontario, Canada) for 10 min before the addition of sevoflurane to the bath. The presynaptic neuron was impaled with a sharp electrode and stimulated to generate 100 action potentials (10 spikes/burst) to allow the uptake of the dye FM1-43 either in the presence or the absence of sevoflurane. FM1-43 and the anesthetic were then replaced with cold saline to prevent neuronal spiking and

thus the subsequent loss of the dye, and to remove background fluorescence. Fluorescence images of the FM1-43-labeled cells were acquired using a Zeiss Axiovert 200 M inverted microscope (Carl Zeiss Canada Ltd.), and images were acquired and processed by excitation filters (480/30 nm), dichroic mirror (505 nm), and emission filters (570 LP nm or 610 nm). Both phase and fluorescent images were captured with a Photometrics Sensys (Photometrics, Tuscon, AZ) 1400 camera (1–100 ms exposure) connected to a computer running Axiovision 3.0 for Windows (Carl Zeiss Canada Ltd.).

Statistical Analysis

All parametric data are expressed as mean \pm SE, and the significance was determined using analyses of variance with repeated measures. Nonparametric data are expressed as percents and were analyzed for significance using the *t* test. Significance was assumed if *P* was less than 0.05.

Results

Sevoflurane Suppresses Cholinergic Synaptic Transmission between VD4 and LPeD1

To test for the effects of inhalation anesthetic sevoflurane on synaptic transmission, specific excitatory synapses between VD4 and its postsynaptic partner LPeD1 were reconstructed in a soma–soma configuration. The isolated somata of VD4 and LPeD1 were extracted from visceral and left pedal ganglia, respectively, and paired overnight²⁷ (fig. 1A). Excitatory, cholinergic synapses similar to those observed *in vivo*^{29,30} developed between the paired cells. Specifically, induced action potentials in VD4 generated 1:1 excitatory postsynaptic potentials (EPSPs) in LPeD1 (*n* = 60; fig. 1B).

To test whether sevoflurane affects synaptic transmission between VD4 and LPeD1, synapses were tested in either the absence or the presence of sevoflurane (0.5–3%). Sevoflurane delivered through a fast perfusion system directly at the contact site²⁸ suppressed synaptic transmission between VD4 and LPeD1 in a concentration-dependent manner (percent of control: 0.5% = 68.81 ± 8.2 , *n* = 9; 1% = 51.30 ± 8.1 , *n* = 6; 1.5% = 43.35 ± 6.7 , *n* = 5). Specifically, the amplitude of VD4-induced EPSPs in LPeD1 was significantly reduced by all sevoflurane concentrations used (fig. 2A). However, an almost complete blockage of synaptic transmission was achieved at a concentration of 3% ($3.13 \pm 4.4\%$ of control, *n* = 5; fig. 2A). In all instances, the synaptic transmission was restored within a few minutes of wash-out with normal saline (fig. 2A, i–iii, and fig. 2B). Together, these data demonstrate that sevoflurane significantly and reversibly blocks synaptic transmission between VD4 and LPeD1 (percent of control: 0.5% = 94.54 ± 4.47 ; 1% = 94.06 ± 1.70 ; 1.5% = 85.53 ± 4.33 ; 3% = 78.73 ± 3.99 ; fig. 2B).

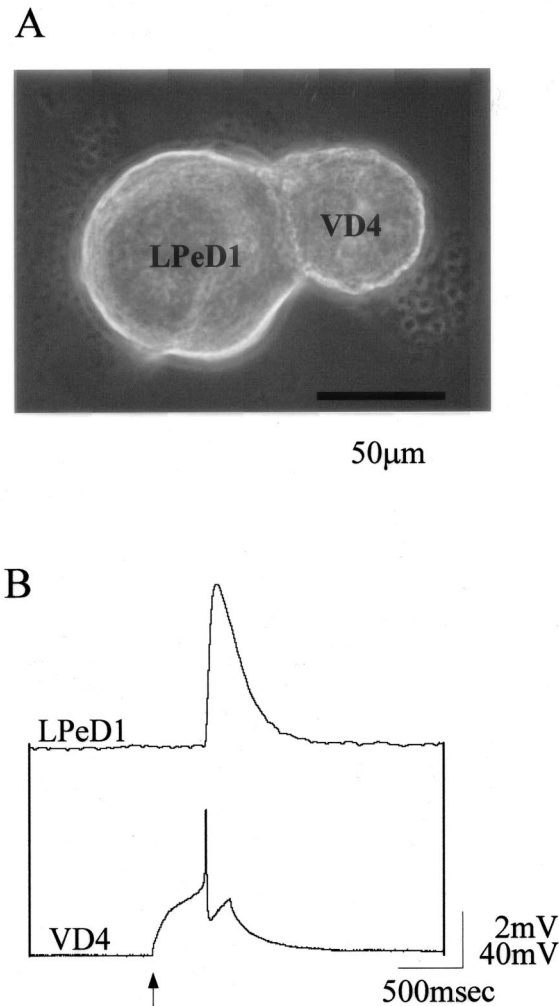


Fig. 1. Synapse formation between soma–soma paired *Lymnaea* neurons. (A) Individually identifiable, presynaptic (visceral dorsal 4 [VD4]) and postsynaptic (left pedal dorsal 1 [LPeD1]) were soma–soma paired overnight. (B) Specific synapses similar to those seen *in vivo* reformed overnight between the paired cells. An action potential in VD4 (at arrow) generated 1:1 excitatory postsynaptic potentials in LPeD1.

Sevoflurane-induced Synaptic Suppression Does Not Involve Presynaptic Secretory Machinery

To test whether sevoflurane-induced suppression of synaptic transmission between VD4 and LPeD1 involved perturbation of presynaptic secretory machinery, cells were paired overnight (fig. 3). After 18–20 h of pairing, intracellular recordings were made from both cells in either the presence or the absence of sevoflurane plus the dye FM1-43. We reasoned that if sevoflurane affected exocytosis or endocytosis after the stimulation of the presynaptic cell, it would not uptake the dye. FM1-43 was added to the culture dish, and images were acquired first in the absence of the presynaptic activity (fig. 3A); no labeling was observed in VD4 under such control conditions (fig. 3B). VD4 was then stimulated by current injections (5 bursts, 100 action potentials), the dye was washed out with normal saline, and further images were

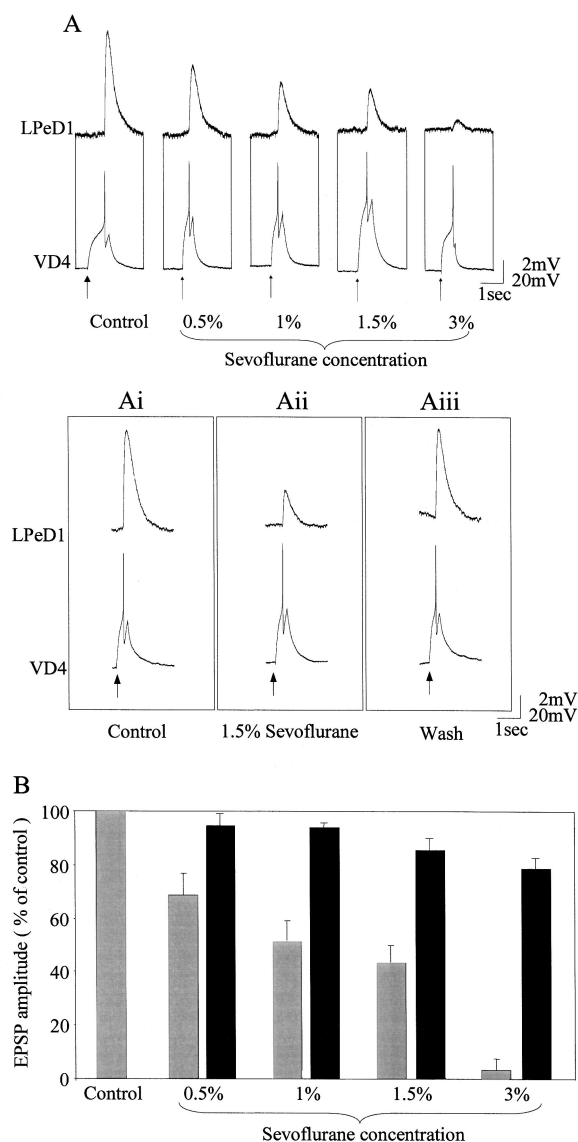


Fig. 2. Sevoflurane blocks synaptic transmission between visceral dorsal 4 (VD4) and left pedal dorsal 1 (LPeD1) in a concentration-dependent manner. VD4 and LPeD1 were somata paired overnight, and synaptic transmission was tested electrophysiologically. Action potentials in VD4 (at arrow) induced 1:1 excitatory postsynaptic potentials (EPSPs) in LPeD1 (A). The amplitude of VD4-induced EPSP in LPeD1 was significantly suppressed by sevoflurane in a concentration-dependent manner (A, *i-iii* show representative traces of sevoflurane (1.5%)–induced suppression of synaptic transmission and subsequently recovery after washout. Sevoflurane-induced effects were concentration dependent, and the synaptic transmission recovered fully after washout with normal saline (B, dark bars). The postsynaptic cell was current clamped at -80 mV throughout the experiment.

acquired. We found fluorescently labeled puncta of VD4 neuritic processes either at the contact site or around the soma of LPeD1 (fig. 3C). Next, to test whether sevoflurane (3%) blocked exocytosis or endocytosis of cholinergic vesicles, images were first acquired in the presence of FM1-43 plus sevoflurane but in the absence of VD4 activity (figs. 3D and E). VD4 was then stimulated

in the presence of sevoflurane plus FM1-43. Similar to the labeling observed under normal conditions (fig. 3C), FM1-43 labeling was discernable at the contact site (fig. 3F), suggesting that sevoflurane affected neither exocytosis nor the endocytotic process. Although these experiments ($n = 6$ /case) do not reveal the qualitative differences between labeling during control (fig. 3C) and anesthetic conditions (fig. 3F), they do suggest that the sevoflurane-induced suppression of synaptic transmission seen previously (fig. 2) may not involve presynaptic machinery.

Sevoflurane Blocks Postsynaptic Cholinergic Response in LPeD1

The synaptic transmission between VD4 and LPeD1 has previously been shown to be cholinergic.^{29,30} To test whether sevoflurane blocks cholinergic response in LPeD1, this neuron was cultured overnight. Intracellular recordings were made, and cholinergic responses were tested either in the presence or absence of various sevoflurane concentrations (0.5–3%). Specifically, cells were current clamped at -65 mV, and acetylcholine (10^{-6} M) was pressure applied under a fast perfusion system²⁸ in either the absence or the presence of sevoflurane. We found that sevoflurane blocked cholinergic responses in LPeD1 in a concentration-dependent (percent of control: 0.5% = 70.88 ± 4.43 ; 1% = 53.88 ± 4.56 ; 1.5% = 37.55 ± 4.89 ; 3% = 5.38 ± 4.83 ; fig. 4A) and reversible manner (washout percent of control: 0.5% = 95.03 ± 3.1 ; 1% = 91.72 ± 2.47 ; 1.5% = 87.46 ± 2.60 ; 3% = 79.43 ± 3.89 ; fig. 4A, *i-iii*, and fig. 4B). Either maximum or almost complete block was observed at a concentration of 3% ($n = 11$ for all concentrations; fig. 4). These data thus show that the sevoflurane-induced suppression of synaptic transmission between VD4 and LPeD1 likely involves postsynaptic cholinergic receptors.

Sevoflurane Does Not Affect Posttetanic Potentiation at the VD4–LPeD1 Synapse

To test for the effects of sevoflurane on short-term synaptic plasticity, synapses were reconstructed overnight as described under the heading “Sevoflurane Blocks Postsynaptic Cholinergic Response in LPeD1.” Simultaneous intracellular recordings were made, and synapses were tested electrophysiologically. After a single action potential in VD4 that generated 1:1 EPSPs in LPeD1, a tetanus (8–10 action potentials) was delivered to VD4. Subsequent action potentials in VD4 delivered within a few seconds of the tetanus resulted in postsynaptic potentiation that only lasted for a few seconds ($n = 20$; fig. 5). To test whether sevoflurane affects this PTP, the above experiment was conducted in the presence of 1.5–3.0% sevoflurane. We discovered that although 1.5% sevoflurane reduced synaptic transmission between VD4 and LPeD1 to approximately 50%, the ratio between

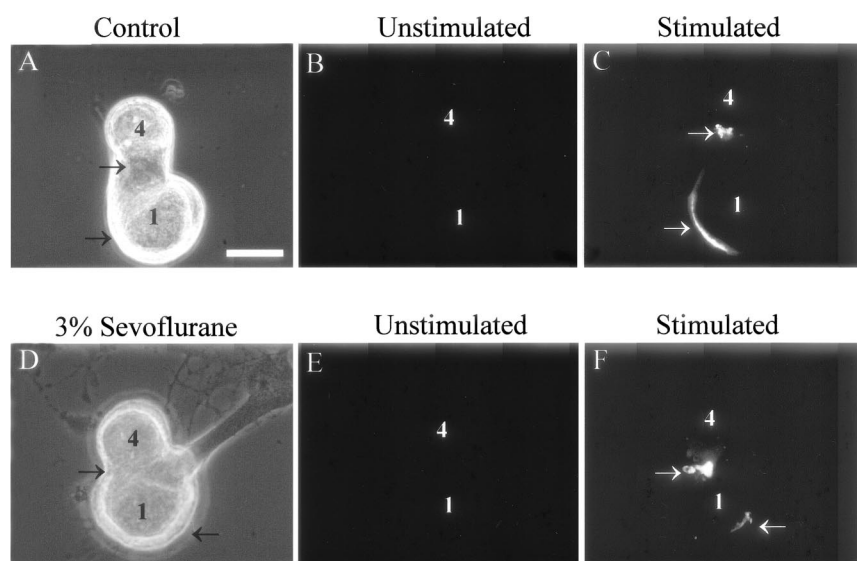


Fig. 3. Sevoflurane does not affect the presynaptic secretory machinery. To test whether sevoflurane-induced suppression of synaptic transmission between visceral dorsal 4 (VD4) and left pedal dorsal 1 (LPeD1) was due to the perturbation of presynaptic release machinery, synapses were tested in either the absence or the presence of 3% sevoflurane (depresses the synapse almost completely) and the dye FM1-43. Neurons were paired overnight and allowed to develop synapses. Synapses developed at the contact site between the pairs, and VD4 processes were seen often unsheathing the LPeD1 somata (arrows, A and D). To demonstrate the pattern of dye localization in a control VD4, the cell was impaled intracellularly, and the dye was added to the preparation. VD4 was prevented from spiking by injecting hyperpolarizing current (0.2 nA) for 10 min, and images were acquired. No staining was discernable in VD4 (B). The presynaptic cell was then stimulated (100 action potentials) by current pulses, and the dye was replaced with normal, cold saline. Images were acquired again, which revealed punctate staining both at the contact site between the cells and also in VD4 processes encircling the LPeD1 somata (arrows, C). The above experiment was repeated in the presence of 3% sevoflurane (D–F). Sevoflurane at a concentration that blocks synaptic transmission almost completely also failed to prevent the uptake of the dye FM1-43, and punctate staining (F) similar to that seen under control (C) conditions was clearly discernable in the presence of this anesthetic.

pretetanic and posttetanic EPSPs did not change (fig. 6), even when the synapse was tested in the presence of 3% sevoflurane (which almost completely blocked the synaptic transmission between the cells; fig. 6B). These data thus demonstrate that sevoflurane does not affect the genesis of PTP at this synapse.

We next sought to determine whether sevoflurane blocked the retention of PTP. The tetanus was delivered to VD4 under control saline conditions, and the preparation was then superfused with either normal saline (fig. 7A) or sevoflurane (fig. 7B) for 5 min. An action potential in VD4 generated an EPSP, whereas a burst produced compound PTP as shown previously (fig. 7). The perfusion was then switched to either normal saline (fig. 7A) or the anesthetic solution (fig. 7B) for an additional 5 min. After 2 min of washout with normal saline, the PTP was then tested as described above. We found that a 5-min exposure to sevoflurane (3%) did not prevent the “expression” of PTP at the VD4–LPeD1 synapse, which exhibited potentiation in a manner similar to that observed under control conditions (posttetanus EPSP amplitude = control: 28.5 ± 2.5 mV; sevoflurane: 27.8 ± 2.3 mV; fig. 7). The amplitudes of the PTP under control and sevoflurane conditions are compared in figure 7. Taken together, these data demonstrate that sevoflurane does not block PTP, nor does its application to a potentiated synapse eliminate short-term plasticity.

Discussion

This study has demonstrated that clinically relevant concentrations of sevoflurane block cholinergic, excitatory synaptic transmission postsynaptically. Moreover, using a model system approach, we have provided the first direct evidence that neither the expression of short-term plasticity nor its retention is affected by sevoflurane. Taken together, our data show that although sevoflurane significantly suppresses synaptic transmission at a cholinergic synapse, it does not affect presynaptic machinery mediating PTP, which, in many other systems, underlies working memory.³¹ This study thus provides direct physiologic evidence for the idea that short-term exposure of synapses to an anesthetic may not affect synaptic plasticity underlying PTP. However, these data should be treated with caution as learning and memory involve a larger population of neurons, often requiring interplay between complex cognitive information processing mechanisms in the brain. These data, at the level of a single synapse, do nevertheless demonstrate that at the cellular level, sevoflurane does not affect short-term synaptic plasticity between VD4 and LPeD1.

Anesthetics agents such as sevoflurane bring about a state of general anesthesia by affecting both excitatory and inhibitory synaptic transmission in the nervous sys-

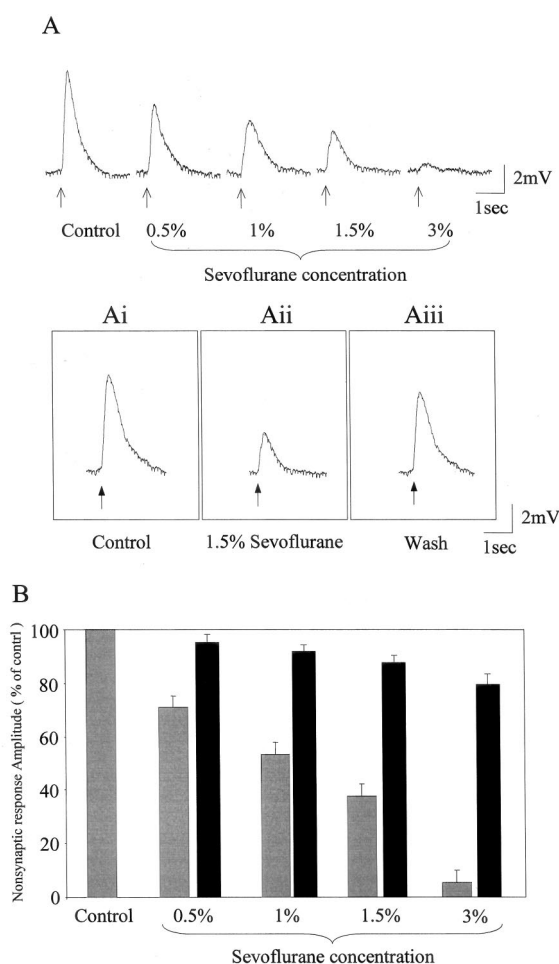


Fig. 4. Sevoflurane blocks cholinergic response in left pedal dorsal 1 (LPeD1). To test whether sevoflurane-induced suppression of synaptic transmission involved cholinergic postsynaptic receptors, acetylcholine was tested on LPeD1 in either the absence or the presence of sevoflurane. Specifically, acetylcholine (10^{-5} M) was pressure applied to a single or paired LPeD1, and its effects were monitored intracellularly, in either the presence or the absence of various sevoflurane concentrations (A). The cholinergic responses in LPeD1 were significantly depressed by sevoflurane in a concentration-dependent and reversible manner (A, *i-iii*). These data are summarized in B, and the darker bars represent washout data.

tem. For example, both glutamatergic and γ -aminobutyric acid-mediated synaptic transmissions are perturbed by intravenous and inhalation anesthetics.¹ The anesthetic-induced changes in the efficacy of synaptic transmission involve either presynaptic or postsynaptic mechanisms or both. In contrast to their effects on glutamatergic and γ -aminobutyric acid-mediated synapses, less understood are the actions of anesthetics on cholinergic synaptic transmission, which, in the central nervous system, is thought to be involved in learning and memory.¹ Similarly, nicotinic acetylcholine receptors in various other brain regions have been implicated in a variety of nervous system functions. For example, basal forebrain neurons involving nicotinic acetylcholine receptors regulate memory and arousal, whereas cholin-

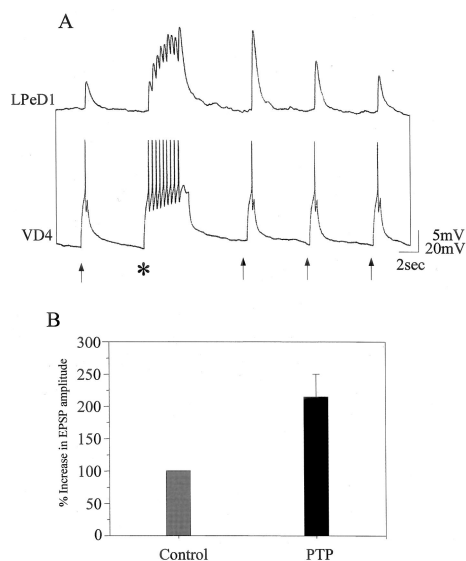


Fig. 5. Visceral dorsal 4 (VD4)-left pedal dorsal 1 (LPeD1) synapse exhibits short-term potentiation. The soma-soma paired cells were simultaneously impaled intracellularly, and synaptic transmission was tested electrophysiologically. (A) Action potentials in VD4 (at arrow) generated 1:1 excitatory postsynaptic potentials (EPSPs) in LPeD1. The amplitude of first EPSP was measured, and a tetanus was delivered to VD4 (at asterisk, 10-10 action potentials), which resulted in a compound postsynaptic potential (PSP) in LPeD1. The subsequent action potential in VD4 (posttetanus) resulted in a few hundred percent enhancement of EPSPs amplitude in LPeD1, which gradually returned to its baseline within seconds. (B) Summary data depicting the percent increase in the amplitude of posttetanic EPSPs in LPeD1.

ergic pathway in pontomesencephalic area regulate sleep, memory, and locomotor patterned activity.^{1,32} Regardless of their location (presynaptic *vs.* postsynaptic), most of these receptors are affected by anesthetics, although their precise sites of action have not yet been defined, because of the complexity of the vertebrate brain. A notable exception is a study on uniquely identified snail neurons where isoflurane was shown to directly inhibit nicotinic acetylcholine receptors with concentration dependencies that were similar to those of mice neurons.^{33,34} Together, the above studies on both vertebrate and invertebrate neurons suggest that anesthetics affect neuronal acetylcholine receptors though their direct actions on "synaptic receptors" have not yet been determined. In this study, we have provided direct evidence that sevoflurane suppresses the function of the synaptic acetylcholine receptors in a concentration-dependent and reversible manner.

Wu *et al.*² have recently demonstrated that isoflurane suppresses neurotransmitter release from glutamatergic, calyx-type synapse in the rat brainstem. These effects were shown to involve an anesthetic-induced reduction in the amplitude of the presynaptic action potential. Similarly, general anesthetics have also been shown to inhibit acetylcholine release in several other preparations,^{35,36} whereas other studies have not detected any

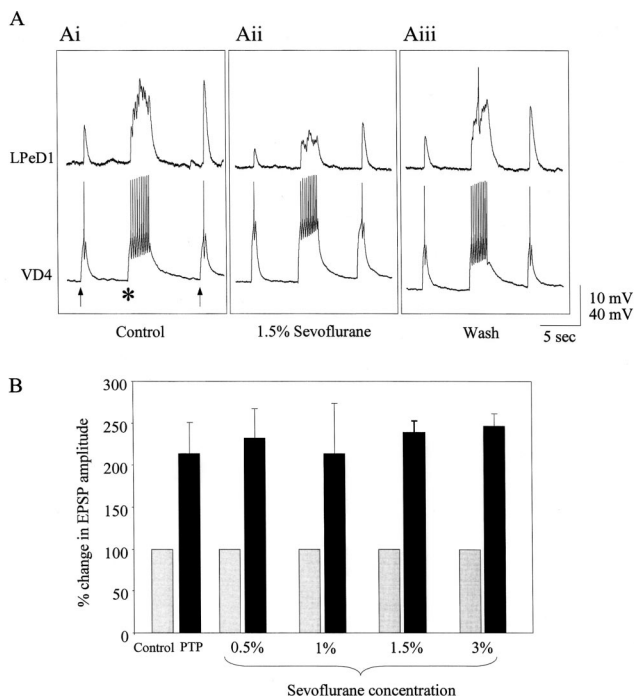


Fig. 6. Sevoflurane does not affect posttetanic potentiation (PTP) at the visceral dorsal 4 (VD4)–left pedal dorsal 1 (LPeD1) synapse. To test whether sevoflurane blocks VD4-induced PTP in LPeD1, synapses were tested electrophysiologically. (A) Action potentials in VD4 (at arrow) generated 1:1 excitatory postsynaptic potentials (EPSPs) in LPeD1 under control (i) and various anesthetic conditions (ii) and after washout (iii). Although VD4-induced EPSPs were significantly suppressed by all sevoflurane concentrations used (A), the ratio between the pretetanus and posttetanus EPSPs did not change significantly (B), even for 3% sevoflurane, which blocked the synaptic transmission almost completely.

affects of halothane, enflurane, or methoxyflurane on the secretion of this transmitter.³⁷ Notwithstanding the fact that these discrepancies may arise from various different approaches or the model system used, this information is important in resolving the issue of whether anesthetics affect learning and memory, arousal, and pain, which often involves cholinergic synaptic transmission. The issue of whether anesthetics affect presynaptic or postsynaptic mechanisms by blocking cholinergic synaptic transmission is difficult to resolve in an intact preparation because cell–cell interactions between defined sets of presynaptic and postsynaptic neurons are often difficult to investigate directly. In this study, we took advantage of an invertebrate model system whose usefulness for various anesthetic studies has been well documented.^{27,33,38–41} Using the well-established soma–soma synapses between identified neurons,^{26–28,38,42} we have previously demonstrated that both inhalation (sevoflurane²⁷) and intravenous (propofol) anesthetics block dopaminergic and cholinergic transmission between the soma–soma paired cells, respectively. In the current study, this model system approach was used to provide direct evidence that clinically relevant concentrations of sevoflurane also

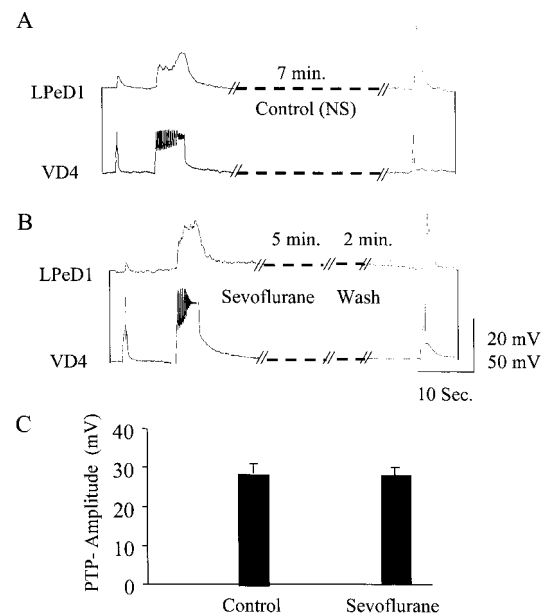


Fig. 7. Sevoflurane does not eliminate posttetanic potentiation (PTP) at the visceral dorsal 4 (VD4)–left pedal dorsal 1 (LPeD1) synapse. To test whether sevoflurane eliminates PTP, the soma–soma synapses between VD4 and LPeD1 were tested electrophysiologically. The PTP paradigm was used as described above. After the tetanus, VD4 was prevented from spiking, and the preparation was exposed to either control saline (A) or sevoflurane (B) for 5 min. The anesthetic solution was replaced with normal saline for 2 min, and the synaptic transmission was tested again under both experimental conditions. The posttetanic action potential in VD4 generated a potentiated excitatory postsynaptic potential in LPeD1 under both experimental conditions; the amplitudes of the posttetanic excitatory postsynaptic potentials were identical (C; control: 28.5 ± 2.5 mV; sevoflurane: 27.8 ± 2.3 mV). NS = not significant.

suppress synaptic transmission between *Lymnaea* neurons paired in a soma–soma configuration. Previous studies on *Lymnaea* have demonstrated that clinically relevant concentrations of halothane (1–2%) induce a state of complete “anesthesia.”⁴³ Moreover, clinically relevant concentrations of enflurane blocked cholinergic synaptic transmission between *Aplysia* neurons,⁴⁴ whereas higher concentrations (4–6%) of sevoflurane were required to block dopaminergic, inhibitory synapses in *Lymnaea*.²⁷ In the current study, we have shown that clinically relevant concentrations of sevoflurane (1–3%) are sufficient to block cholinergic synaptic transmission between VD4 and LPeD1. Our data are thus consistent with previous studies on invertebrate models, and together, they demonstrate that clinically relevant concentrations effectively block/suppress synaptic transmission between neurons.

Fluorescent labeling of the presynaptic vesicles with the dye FM1-43 strongly suggests that sevoflurane most likely does not affect exocytotic or endocytotic processes—these data do not, however, provide unequivocal evidence to this effect. Specifically, we could not precisely quantify the extent of fluorescent labeling with FM1-43 in either the absence or the presence of sevoflu-

rane. Nevertheless, these results demonstrate that sevoflurane does not significantly suppress exocytosis and endocytosis of cholinergic vesicles. These data are also consistent with our previously published studies in which, using FM1-43 dye, we demonstrated that propofol also did not affect both exocytosis and endocytosis between the soma-soma paired cells.³⁸ Wu *et al.*,² on the other hand, demonstrated that isoflurane-induced suppression of synaptic transmission at the calyx-type mammalian synapses involves presynaptic sites, such as the Na⁺ channels. Because in our previous work and the data presented in this study, we did not observe an anesthetic-induced reduction in the amplitude of the presynaptic action potential, we are confident that in our model, sevoflurane does not affect presynaptic machinery such as the Na⁺ channels or the vesicular endocytosis/exocytosis. Moreover, because extrasynaptic, cholinergic responses in LPeD1 neurons were completely and reversibly blocked by sevoflurane, it seems safe to infer that the suppression of synaptic transmission between VD4 and LPeD1 may have primarily involved postsynaptic mechanisms. Consistent with this notion are previous studies on unidentified *Lymnaea* where acetylcholine receptors were also shown to be blocked by another inhalation anesthetic.^{33,34} However, whether these anesthetic-induced effects on cholinergic receptors involved any specific, postsynaptic ion channels or receptors remains unknown and will require further investigation. In vertebrate models, the neuronal nicotinic acetylcholine receptors have been shown to exhibit greater sensitivities to inhalation anesthetics as compared with their muscle counterparts.^{7,8} Although the mechanisms underlying these differential responses remain undefined, similar comparative data in invertebrates await further identification and characterization of various types of acetylcholine receptors.

In contrast to their actions on synaptic transmission, much less understood are the effects of anesthetics on synaptic plasticity that forms the basis for learning and memory in various animal models. For example, although inhalation anesthetics have been shown to block long-term potentiation at hippocampal synapses,^{45,46} their effects on short-term potentiation mediating working memory have not yet been fully defined. The data presented in this study thus provide the first direct evidence that clinically used concentrations of sevoflurane do not affect the "expression" of PTP, nor do they eliminate the short-term plasticity that is induced in the absence of this anesthetic. The synaptic transmission between the paired cells was significantly reduced, although the ratio between presynaptic and postsynaptic EPSPs remained unperturbed by sevoflurane. Because sevoflurane exposure of the synapse, after the PTP, had no effect on posttetanic EPSPs, our data provide direct evidence that this volatile anesthetic does not eliminate

PTP, which had otherwise developed under normal conditions.

We have previously demonstrated that the PTP at VD4 and LPeD1 synapse primarily involves presynaptic mechanisms⁴⁷ and is not time dependent but rather use dependent (Naruo *et al.*, unpublished data). Specifically, if VD4 is stimulated to fire an action potential after the tetanus, the synapse depotentiates, and the synaptic transmission returns to its baseline. However, if VD4 is prevented from firing, the synapses remains potentiated for up to several hours.⁴⁸ This synapse thus exhibits synaptic characteristics, which can account for working memory. Consistent with this notion are the data presented in figure 7, which shows that in the absence of VD4 activity, the synapse had remained potentiated for several minutes during sevoflurane exposure. Thus, PTP was shown here to be unaffected by sevoflurane. In this study, we have also shown that the sevoflurane-induced suppression of synaptic transmission between VD4 and LPeD1 primarily involves postsynaptic acetylcholine receptors, thus validating our hypothesis that sevoflurane-induced suppression of synaptic transmission between VD4 and LPeD1 involves postsynaptic but not presynaptic mechanisms. This model also provides us with an additional tool (sevoflurane) to decipher the cellular and synaptic mechanisms of synaptic plasticity in this and the other models.

The neurons used in the current study comprise the cardiorespiratory central pattern generator that underlies aerial respiration in *Lymnaea*,²³ which exhibits various forms of memory.^{49–51} Because both the behavioral and the neuronal components of this memory have been extensively characterized at the level of single neurons, we believe that elucidating the mechanisms by which sevoflurane affects synaptic potentiation will elucidate the neuronal basis of behavioral plasticity at a resolution unapproachable elsewhere.

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