

# Effects of Halothane on Excitatory Neurotransmission to Medullary Expiratory Neurons in a Decerebrate Dog Model

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**Background:** The activity of canine expiratory (E) neurons in the caudal ventral respiratory group is primarily dependent on *N*-methyl-D-aspartic acid (NMDA) receptor-mediated excitatory chemodrive inputs and modulated by an inhibitory mechanism mediated via  $\gamma$ -aminobutyric acid<sub>A</sub> (GABA<sub>A</sub>) receptors. In an intact canine preparation, halothane depressed the activity of these neurons mainly by reduction in overall glutamatergic excitation. A new decerebrate preparation allows comparison of the effects of halothane on these synaptic mechanisms with an anesthetic-free baseline state.

**Methods:** Two separate studies were performed in decerebrate, vagotomized, paralyzed, mechanically ventilated dogs during hypercapnic hyperoxia. In study 1, the effect of 1 minimum alveolar concentration (MAC) halothane on extracellularly recorded E neuronal activity was studied before and during complete GABA<sub>A</sub> receptor blockade by localized pressure ejection of bicuculline. Complete blockade of the inhibitory mechanism allowed differentiation between the effects of halothane on overall GABA<sub>A</sub>-mediated inhibition and on overall NMDA receptor-mediated excitation. In study 2, the effect of 1 MAC halothane on the dose response of neurons to localized picroejection of the glutamate agonist NMDA was used to estimate halothane effect on postsynaptic glutamatergic excitatory neurotransmission.

**Results:** In study 1, the spontaneous activity of 14 E neurons was depressed  $38.6 \pm 20.6\%$  (mean  $\pm$  SD) by 1 MAC halothane. Overall excitation was depressed  $31.5 \pm 15.5\%$ . The GABAergic inhibition showed a  $11.7 \pm 18.3\%$  enhancement during halothane. In study 2, the spontaneous activity of 13 E neurons was again significantly depressed by 1 MAC halothane ( $27.9 \pm 10.6\%$ ), but the postsynaptic response of the neurons to exogenous NMDA was not significantly depressed by halothane ( $3.3 \pm 38.4\%$ ).

**Conclusions:** Together these results suggest that in our E neuron paradigm, halothane exerted its depressive effect mainly via reduction of glutamatergic presynaptic mechanisms. (Key words: Brain stem; ionotropic neurotransmitter; volatile anesthetics.)

EXPIRATORY (E) bulbospinal premotorneurons located in the caudal ventral respiratory group (cVRG) provide

the major input for motoneurons of the expiratory musculature.<sup>1</sup> We have previously shown that in the vagotomized dog the excitatory drive of cVRG E neurons, *i.e.*, mainly central and peripheral chemoreceptors, is primarily *N*-methyl-D-aspartic acid (NMDA) receptor mediated.<sup>2</sup> Discharge activity is modulated solely by a  $\gamma$ -aminobutyric acid<sub>A</sub> (GABA<sub>A</sub>)ergic inhibitory input.<sup>3</sup>

Increasing halothane concentrations between 1-2.5 minimum alveolar concentration (MAC) reduced neuronal activity in a linear fashion.<sup>4</sup> We showed that in a neuraxis intact preparation this depression is caused mainly by a reduction of glutamatergic excitatory neurotransmission rather than by enhancement of GABA<sub>A</sub>ergic inhibition.<sup>5</sup>

A shortcoming of that preparation was that the lack of an anesthetic-free control state did not allow the study of the effects of the volatile anesthetic *per se*. One common approach for *in vivo* studies of neurophysiologic mechanisms is the use of decerebrate animal models provided that the neuronal circuits of interest remain largely intact. Traditionally, feline decerebrate preparations have been used for brain stem respiratory studies because, as a result of its unique intracerebral circulation, decerebration in cats is relatively simple.<sup>6,7</sup> In comparison, the dog has a much more extensive circle of Willis that can cause significant blood loss and an unstable preparation.

We recently developed a decerebration procedure that resulted in a stable decerebrate canine preparation. This preparation showed a preserved respiratory pattern and peak neuron activities similar to the intact preparation. We used this decerebrate model to study the effect of halothane on overall GABAergic and glutamatergic neurotransmission analogous to the neuraxis intact preparation.<sup>5</sup>

In a second study, we sought to differentiate excitatory neurotransmission into its presynaptic and postsynaptic components by assessing the effect of halothane on the postsynaptic NMDA receptor.

## Materials and Methods

### Animal Preparation and General Methodology

This research was approved by the Medical College of Wisconsin Animal Care Committee, Milwaukee, Wisconsin, and conformed to standards set forth in the National Institutes of Health Guide for Care and Use of Laboratory Animals, Bethesda, Maryland.

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Dogs were induced by mask with halothane and intubated with a cuffed endotracheal tube. The preparation has been described elsewhere in detail.<sup>5</sup> Briefly, after bilateral vagotomy and bilateral pneumothorax to achieve peripheral deafferentation, an occipital craniotomy was performed to expose the medulla oblongata, and the phrenic nerve was prepared for recording. Phenylephrine ( $0.5\text{--}5\ \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was usually required during the anesthetic runs to keep the mean arterial pressure above 75 mmHg. Protocols were performed only during steady-state conditions for blood pressure. Esophageal temperature was maintained at  $37.5\text{--}38.5^\circ\text{C}$ .

After completion of the occipital craniotomy, the animals were decerebrated as described by Tonkovic-Capin *et al.*<sup>8</sup> Briefly, the procedure leads to an anatomically well-defined mid-collicular decerebration with modest blood loss (10 ml/kg). After completion of the decerebration, the anesthetic was discontinued. Pancuronium,  $0.1\ \text{mg/kg}$  followed by  $0.1\ \text{mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ , was then given to avoid motion artifacts during neuronal recordings.

#### *Neurotransmitter Pressure Picoejection and Neuron Recording Technique*

Expiratory neurons of the cVRG were located as described elsewhere.<sup>4,9</sup> Multibarrel compound glass electrodes consisting of a recording barrel containing a  $7\text{-}\mu\text{m}$  carbon filament and three drug barrels were used to simultaneously record extracellular neuronal action potential activity before and during pressure ejection of respective neurotransmitters onto the neuron of interest.<sup>2,3,5</sup> We used the NMDA receptor agonist *N*-methyl-D-aspartate ( $200\ \mu\text{M}$ ; Research Biochemicals, Natick, MA) and the GABA<sub>A</sub> receptor antagonist bicuculline methyl chloride (BIC,  $200\ \mu\text{M}$ ; Research Biochemicals), which were dissolved in an artificial cerebrospinal fluid. The artificial cerebrospinal fluid consisted of: 124 mM NaCl, 2 mM KCl, 2 mM MgCl<sub>2</sub>, 1.3 mM KH<sub>2</sub>PO<sub>4</sub>, 0.9 mM CaCl<sub>2</sub>, 26 mM NaHCO<sub>3</sub>, and 11 mM glucose, and the pH was adjusted to 7.2–7.4 by aeration with 5% CO<sub>2</sub>/95% O<sub>2</sub>. The drug-free artificial cerebrospinal fluid vehicle was routinely used as a control to demonstrate lack of vehicle effect (an example of such a drug-free artificial cerebrospinal fluid vehicle run is illustrated in our previous publication<sup>5</sup>).

#### *Data Recording and Collection*

Extracellular activity from individual E neurons, phrenic nerve activity, a picoejection marker, airway carbon dioxide and volatile anesthetic concentration, systemic blood pressure, and airway pressure were recorded on a digital tape system. These variables or their time averages were also continuously displayed on a polygraph during the experiments. Timing pulses at the beginning and end of neural inspiration were derived from the phrenic neurogram and were used to aid in data

analysis. The tape-recorded data were digitized and analyzed offline.

#### *Experimental Protocols*

A minimum of 1 h was allowed for preparation stabilization before data collection. All experiments were performed under hyperoxic (inspired oxygen fraction > 0.8) and steady-state hypercapnic conditions (target partial pressure of alveolar carbon dioxide [Paco<sub>2</sub>], 50–60 mm Hg). This level of hypercapnia was chosen to ensure adequate phrenic nerve activity during halothane application and to allow comparison with our previous studies. Target Paco<sub>2</sub> was held constant for all experimental runs within an animal, but was allowed to differ within the set target range among animals.

#### *Protocol Study 1: Effects of Halothane on Overall Synaptic Neurotransmission*

After a stable signal from an E neuron was obtained the neuronal activity was continuously recorded throughout the entire protocol. The peak neuronal discharge frequency ( $F_n$ ) was measured for 10–20 respiratory cycles during a preejection control period ( $F_{\text{con}}$ ). Then the GABA<sub>A</sub> antagonist BIC was picoejected until complete block of GABAergic inhibition occurred, *i.e.* until an increase in dose rate did not achieve any further increase in discharge frequency. Typically, picoejection durations of 5–10 min with increasing dose rates were required. After this run, complete postejction recovery was awaited (30–45 min). Thereafter, the anesthetic was introduced to a depth of 1 MAC halothane (0.9 vol%), and after an equilibration time of 20 min, the same procedure was repeated. Peak dose rates during anesthesia always had to match or exceed those during the 0-MAC run.

The complete protocol, including the 0-MAC level, the anesthetic wash-in, the 1-MAC level, and the return to 0-MAC level (anesthesia-free end control), required about 4 h.

#### *Statistical Analysis Study 1*

The method of analysis was the same as in our previous publication.<sup>5</sup> All data were normalized to the peak activity of the neuron during maximal BIC block at the 0-MAC level. This represented the maximal discharge frequency for each neuron and was assigned a value of 100%. A two-way, repeated measures analysis of variance with main factors of level of anesthesia (0 or 1 MAC) and neurotransmitter concentration (preejection control *vs.* maximal BIC block) was used.

To determine the prevailing GABA<sub>A</sub>ergic inhibition, we defined the peak discharge frequency of the neuron ( $F_n$ ) for the preejection control period,  $F_{\text{con}}$ , and for the maximal frequency under BIC block ( $F_c$ ).  $F_c$  is thus a measure of the total excitatory drive to the neuron,

**Table 1. Protocol and Calculations Used to Separate Overall Excitatory and Inhibitory Components of Synaptic Mechanisms**

Anesthetic Level	Measurement of Synaptic Inputs	Variables	Picoejection Procedure
0 MAC	Excitatory	$F_{e0}$	Inhibitory antagonist GABA <sub>A</sub> block (BIC)
	Inhibitory	$\alpha_0$	
1 MAC	Excitatory	$F_{e1}$	Inhibitory antagonist GABA <sub>A</sub> block (BIC)
	Inhibitory	$\alpha_1$	
Calculations for anesthetic-induced changes		Overall excitatory component	Eq. 1: $\Delta F_e = (F_{e1} - F_{e0})/F_{e0}$
		Overall inhibitory component	Eq. 2: $\Delta\alpha = (\alpha_1 - \alpha_0)/\alpha_0$

MAC = minimum alveolar concentration; GABA<sub>A</sub> =  $\gamma$ -aminobutyric acid receptor type A; BIC = bicuculline; eq. = equation;  $\alpha = [F_e - F_{con}]/F_e$ ;  $F_e$  = peak discharge frequency of the neuron during complete GABA<sub>A</sub>ergic block with BIC;  $F_{con}$  = control peak discharge frequency during the prevailing basal conditions of GABA<sub>A</sub>ergic inhibition (the level of prevailing GABA<sub>A</sub>ergic inhibition is described by the inhibitory constant  $\alpha$ —see text for details).

whereas  $F_{con}$  represents this drive reduced by the prevailing basal GABAergic inhibition.

We described the level of prevailing GABAergic inhibition by the inhibitory constant  $\alpha$  and defined it as:  $\alpha = [F_e - F_{con}]/F_e$ . The values for  $F_e$  and  $F_{con}$  were obtained for the 0-MAC level ( $F_{con0}$ ,  $F_{e0}$ ) and the 1-MAC level ( $F_{con1}$ ,  $F_{e1}$ ) in the experimental protocols. These were then used in the calculation of anesthetic-induced effects on overall excitatory ( $\Delta F_e$ ) and overall inhibitory ( $\Delta\alpha$ ) neurotransmission, as described in equations 1 and 2 of table 1.

#### Protocol Study 2: Effects of Halothane on Postsynaptic Glutamatergic Excitation

The peak neuronal discharge frequency ( $F_n$ ) was measured for 10–20 respiratory cycles during the preejection control period ( $F_{con}$ ). Then the glutamate receptor agonist NMDA was applied in increasing dose rates until an increase of neuronal frequency of at least 40 Hz was achieved. Typically, picoejection durations of 6–8 min with two to three dose rates were needed. Post-ejection recovery was achieved within 5 min. The run was repeated at the 1-MAC level and again at the 0-MAC end-control level.

#### Statistical Analysis Study 2

The effect of the glutamatergic agonist NMDA on E neuron frequency was quantified in the following manner: The picoejection dose rate that caused a 40-Hz net increase in neuron activity in the absence of halothane was determined from the NMDA dose-response curve and designated  $D_{40Hz}$ . To confirm the linearity of the NMDA dose-response curves in this range, the net increase at one half the  $D_{40Hz}$  dose rate was determined and designated  $\frac{1}{2} D_{40Hz}$ . The corresponding net increases of neuronal activity then were determined for 1 MAC halothane. All neuron response data were then normalized to the 40-Hz net increase at 0 MAC, which was assigned a value of 100%. A two-way, repeated measures analysis of variance with main factors level of anesthesia (0 or 1 MAC halothane) and neurotransmitter status (preejection control *vs.* NMDA response) was used.

All data values are given as mean  $\pm$  SD, and  $P < 0.05$  was used to indicate significant differences unless stated otherwise.

## Results

Typically, one complete protocol, consisting of 0-MAC, 1-MAC, and 0-MAC end-control levels, could be obtained per animal. Data from animals with incomplete protocols were not included.

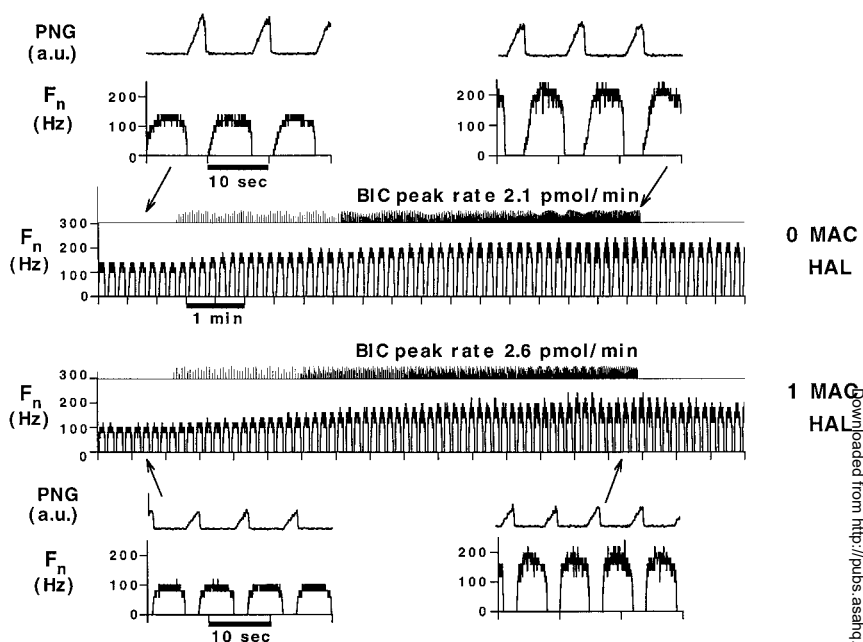
#### Study 1: Effects of Halothane on Overall Synaptic Neurotransmission

Seventeen animals were studied, and complete protocols were obtained in 14 dogs. Figure 1 shows a representative example of a BIC protocol. During both anesthetic states (0 and 1 MAC), complete BIC block caused approximately a doubling of the neuron baseline activity: At 0 MAC halothane, maximal BIC increased peak  $F_e$  from 133 to 227 Hz, yielding an inhibitory factor of  $\alpha_0 = [F_{e0} - F_{con0}]/F_{e0}$ , *i.e.*,  $\alpha_0 = (227 - 133)/227 = 0.42$  (fig. 2). This means that the GABAergic inhibition attenuated the neuronal output by 42%. The corresponding peak  $F_e$  values for 1 MAC halothane were 100 and 183 Hz, yielding an  $\alpha_1 = 0.46$ . Thus, for this neuron, 1 MAC halothane enhanced the tonic inhibition by 9.7% [*i.e.*,  $\Delta\alpha = (\alpha_1 - \alpha_0)/\alpha_0 = (0.46 - 0.42)/0.42 = 0.097$ ].

At the same time, the excitatory activity, which was measured in the absence of inhibition (*i.e.*, during maximal BIC block) was reduced by 19%. Thus, excitatory synaptic drive was reduced by the anesthetic, whereas the inhibitory drive was slightly enhanced. In accordance, control peak discharge frequency,  $F_{con}$ , was reduced by 25%.

The pooled data from 14 neuron protocols with end-control runs are shown in figure 3. Analysis of the pooled data, as exemplified in figure 2, shows that 1 MAC halothane caused a small but significant increase in the  $\alpha$  values (from  $\alpha = 0.54 \pm 0.11$  at 0 MAC to  $0.59 \pm 0.12$  at 1 MAC). This represents a  $11.7 \pm 18.3\%$  enhancement in overall GABAergic inhibition ( $\Delta\alpha$ ; fig. 3, bottom middle bar).

**Fig. 1.** Response of an expiratory neuron to picoejection of the GABA<sub>A</sub> antagonist bicuculline (BIC) at 0 and 1 minimum alveolar concentration (MAC) halothane (HAL). The picoejection response curves to BIC (see picoejection marker) are shown in the middle traces as rate-meter recordings of the neuronal discharge frequency (F<sub>n</sub>). The top and bottom records show time-expanded views of the phrenic neurogram (PNG; arbitrary units [a.u.]) and simultaneous neuronal rate-meter recordings during both the preejection control period (left traces) and maximal increase of the neuronal discharge frequency (right traces).



At the same time, 1 MAC halothane caused a significant  $31.5 \pm 15.5\%$  decrease in overall excitation ( $\Delta F_e$ ; fig. 3, bottom left bar). Peak  $F_{con}$  was significantly depressed from  $46.3 \pm 10.8$  to  $27.5 \pm 9.2$  ( $F_{con}$ ; fig. 3, upper bar), or by  $38.6 \pm 20.6\%$  ( $\Delta F_{con}$ ; fig. 3, bottom right bar) by 1-MAC halothane.

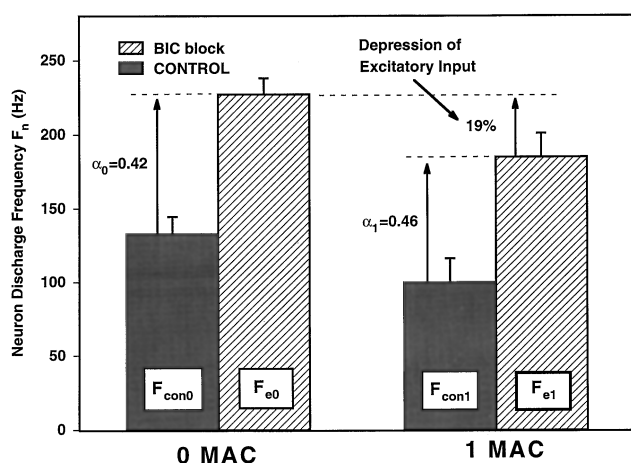
*Study 2: Effects of Halothane on Postsynaptic Glutamatergic Excitation*

Sixteen animals were studied, and 13 complete protocols were obtained. Figure 4 shows a representative example of an E neuronal response to picoejection of

NMDA before and during 1 MAC halothane. At 1 MAC halothane,  $F_{con}$  was reduced from 188 to 145 Hz (fig. 4, preejection period, and fig. 5, left). In this example,  $D_{40Hz}$  at 0 MAC was 3.9 pM NMDA/min. The net frequency increase at  $D_{40Hz}$  at 1 MAC halothane was 39.9 Hz (fig. 5, right). The  $\frac{1}{2} D_{40Hz}$  values were 23.2 Hz at 0 MAC halothane and 21.2 Hz at 1 MAC halothane (fig. 5, left and right).

The pooled normalized baseline data for 13 complete neuron protocols (0 MAC – 1 MAC – 0 MAC) of study 2 again show that halothane significantly depresses  $F_{con}$  to  $27.9 \pm 10.6\%$  (data not shown). This level of depression is not statistically different from the  $38.6 \pm 20.6\%$  depression of  $F_{con}$  found in study 1 (unpaired *t* test). End-control  $F_{con}$  was slightly depressed to  $91.8 \pm 5.0\%$ . This may suggest incomplete recovery or a small deterioration of the preparation over time.

The pooled NMDA net increase data are summarized in figure 6, top, and the normalized data are shown in figure 6, bottom. halothane reduced the net increase from 100% to  $96.7 \pm 38.4\%$  at  $D_{40Hz}$ , which is not statistically significant (fig. 6, lower right hatched bar). The 0-MAC end-control value for  $D_{40Hz}$  was  $108 \pm 48.8\%$ , which was not significantly different from the 0-MAC control. The corresponding normalized  $\frac{1}{2} D_{40Hz}$  data were not statistically different (fig. 6, lower left). Slope analysis for  $\frac{1}{2} D_{40Hz}$  and  $D_{40Hz}$  values confirmed linearity over the full dose range.



**Fig. 2.** Method used to quantify the effects of halothane on overall synaptic neurotransmission (data from neuron shown in fig. 1). Values for peak neuronal discharge frequency (F<sub>n</sub>) before bicuculline (BIC) block (F<sub>con</sub>) and during BIC block (F<sub>e</sub>) at each minimum alveolar concentration (MAC) level were used to calculate the inhibitory factor  $\alpha$ . During BIC block ( $\alpha = 0$ ), 1 MAC halothane reduced the overall excitatory drive by 19%. Coefficient of variations are (left to right): 1.1, 2.0, 1.5, and 2.7%.

*Effects of Halothane on Phrenic Activity and Respiratory Rate*

The peak phrenic nerve activity was significantly depressed by 1 MAC halothane. Peak phrenic nerve activity was reduced by  $47.8 \pm 19.8\%$  (mean  $\pm$  SD) by 1 MAC

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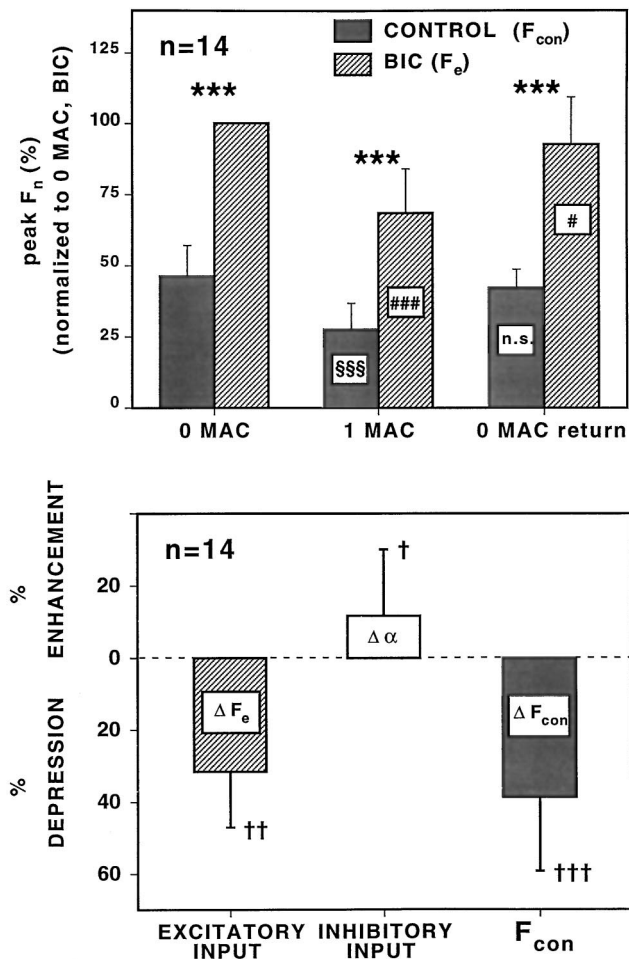


Fig. 3. Pooled summary data of study 1. (Top) Mean values  $\pm$  SD of normalized peak neuronal discharge frequency ( $F_n$ ; %) before ( $F_{con}$ ) and during ( $F_e$ ) maximal GABAergic block with bicuculline (BIC) for the 0 minimum alveolar concentration (MAC), 1 MAC, and 0 MAC end-control doses for 14 neurons. At all MAC levels,  $F_e$  was significantly greater than  $F_{con}$  (\*\* $P$  < 0.001). Halothane, 1 MAC, caused a significant reduction of  $F_{con}$  (SSS $P$  < 0.001) and  $F_e$  (### $P$  < 0.001). The 0-MAC end-control values for  $F_{con}$  were not different (not significant) from the initial 0 MAC values, indicating stability of the preparation. A small but significant decline in  $F_e$  ( $7.4 \pm 16.7\%$ ) of the end control was present ( $\#P$  < 0.05). (Bottom) Mean depression  $\pm$  SD of overall excitatory drive, ( $\Delta F_e$ ), mean enhancement of inhibitory neurotransmission ( $\Delta \alpha$ ), and the resulting mean depression of control frequency ( $\Delta F_{con}$ ) by 1 MAC halothane in 14 neurons. ( $\dagger P$  < 0.05,  $\dagger\dagger P$  < 0.01,  $\dagger\dagger\dagger P$  < 0.001 relative to no change.)

halothane, whereas neural respiratory rate increased from  $15.4 \pm 8.4$  to  $23.2 \pm 13.2$  breaths/min.

## Discussion

Results of the current studies in a decerebrate canine preparation lead to the conclusion that the depressive effect of halothane on cVRG E neurons *in vivo* is predominantly the result of a presynaptic reduction of glutamatergic excitatory neurotransmission. Our methodol-

ogy, however, does not allow us to pinpoint specific presynaptic sites.

The lack of effect of halothane on postsynaptic glutamatergic neurotransmission (e.g., fig. 6) is in agreement with the *in vitro* studies by Perouansky *et al.*<sup>10</sup> Using whole-cell patch clamp recordings in CA1 pyramidal cells in mouse hippocampal slices, they showed that halothane depressed glutamate receptor-mediated excitatory postsynaptic potentials evoked by stimulation of afferent fibers. Halothane did not influence either the NMDA receptor-mediated or the non-NMDA receptor-mediated excitatory postsynaptic potentials evoked by bath application of the respective agonists and did not alter the receptor kinetics. The effect of halothane thus occurred most likely by inhibition of presynaptic glutamate release.<sup>10</sup> In support of this possible mechanism halothane has been shown to reduce glutamate release from cerebral synaptosomes.<sup>11</sup>

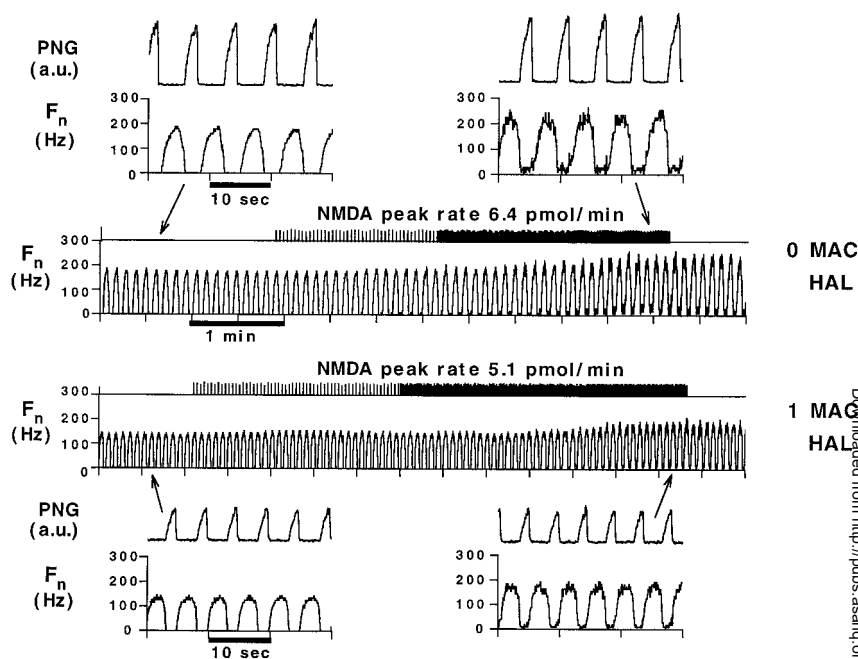
We found a small but significant enhancement of overall GABAergic inhibition at 1 MAC halothane. This is in general agreement with recent *in vitro* studies on the effects of inhalational anesthetics on the miniature inhibitory postsynaptic currents in CA1 pyramidal neurons.<sup>12</sup> Halothane reduced the amplitude but increased the duration of the miniature inhibitory postsynaptic currents, which suggests a reduction in the presynaptic release of GABA but an enhanced postsynaptic action of GABA.<sup>12</sup> The overall effect was a greater than 100% increase in the total charge transferred during the miniature inhibitory postsynaptic currents, or net increase in GABAergic inhibition. With regard to our E neuronal study, it is possible that presynaptic GABAergic mechanisms were depressed in a manner similar to that of glutamate. The combination of reduced presynaptic transmitter release with an enhanced postsynaptic action could have resulted in the small overall GABAergic enhancement we observed.

Our previous data in a neuraxis-intact canine preparation, however, showed that a 1-MAC increase in halothane, from 1 MAC to 2 MAC, produced a  $33.5 \pm 17.2\%$  reduction in overall GABAergic inhibitory neurotransmission.<sup>5</sup> Although we sought to resolve this issue in our decerebrate preparation, we were unable to do so, because most animals became apneic at levels higher than 1.5 MAC halothane, even in the presence of significant hypercapnia.

It thus seems to be necessary to view modification of overall excitation and inhibition beyond synaptic actions. The observed apnea at a lower halothane level than in the intact preparation suggests that in the decerebrate preparation a suprapontine descending excitatory input to the rhythm-generation network has been eliminated.

Consideration of the functional network also helps to explain the seemingly contradictory effects of 1 and 2 MAC halothane on overall GABAergic inhibition. Perouansky *et al.*<sup>13</sup> have shown that in a mouse hippocam-

**Fig. 4.** Response of an expiratory neuron to picoejection of the *N*-methyl-D-aspartic acid (NMDA) receptor agonist NMDA at 0 minimum alveolar concentration (MAC) and 1 MAC halothane (HAL). The picoejection response curves to NMDA (see picoejection marker) are shown in the middle traces as rate-meter recordings of the E neuronal discharge frequency ( $F_n$ ). Arrows: Time-expanded views of the phrenic neurogram (PNG; arbitrary units [a.u.]) and  $F_n$  are shown for 0 MAC (upper) and 1 MAC (lower) halothane levels (see text for details). Respiratory rate increased from 10 to 15 breaths/min during halothane application.



pal slice preparation halothane dose dependently depressed the glutamatergic excitation of inhibitory interneurons. An equivalent of 2 MAC halothane depressed the excitation of the interneuron by 50% and consequently would have reduced the inhibition of the secondary pyramidal cell.

Banks and Pearce<sup>12</sup> showed for CA1 pyramidal neurons that the increase in postsynaptic GABA receptor function by volatile anesthetics peaked at the equivalent of 1 to 2 MAC. We speculate that in our *in vivo* preparation at 1 MAC halothane, a near-maximal receptor-mediated increase in inhibition was present and accounted for the increase in overall inhibition to the E neurons. At 2 MAC halothane, however, the anesthetic may have decreased the presynaptic component of in-

hibitory input to an extent that the net effect was reduction of overall inhibition.

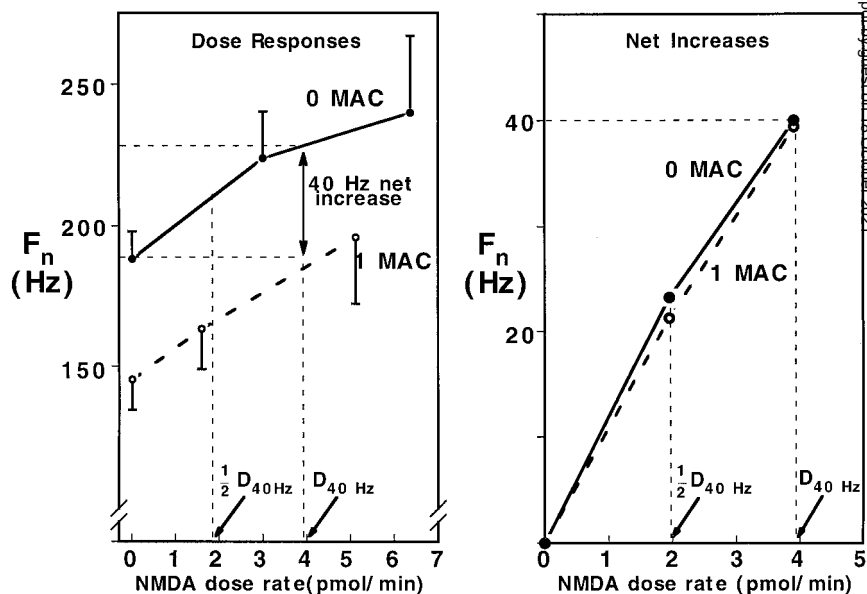
Clearly, additional studies are warranted to determine the relative magnitude of presynaptic and postsynaptic GABAergic effects of halothane.

*Methodologic Considerations*

We have pointed out some of the limitations of our methodology in our previous publication<sup>5</sup> and a detailed discussion is provided elsewhere.<sup>3,14</sup> The decerebrate canine preparation was developed to study the effect of volatile anesthetics *per se* on respiratory neurotransmission *in vivo*.

We found that respiratory patterns were similar to those in the intact animal. Decerebrate, vagotomized

**Fig. 5.** Method used to analyze the effect of halothane on postsynaptic *N*-methyl-D-aspartic acid (NMDA)-induced excitation of E neurons (data from neuron shown in fig. 4). The NMDA dose rate that resulted in a 40-Hz net increase in peak neuronal discharge frequency ( $F_n$ ) at 0 minimum alveolar concentration (MAC) halothane ( $D_{40Hz}$ ) was determined by interpolation from the dose-response curve (left panel).  $D_{40Hz}$  was 3.9 pm/min. At this dose rate, NMDA increased peak  $F_n$  at 1 MAC halothane by 39.4 Hz (see right panel for net increases). The  $1/2 D_{40Hz}$  values were determined from the dose-response curves by interpolation.



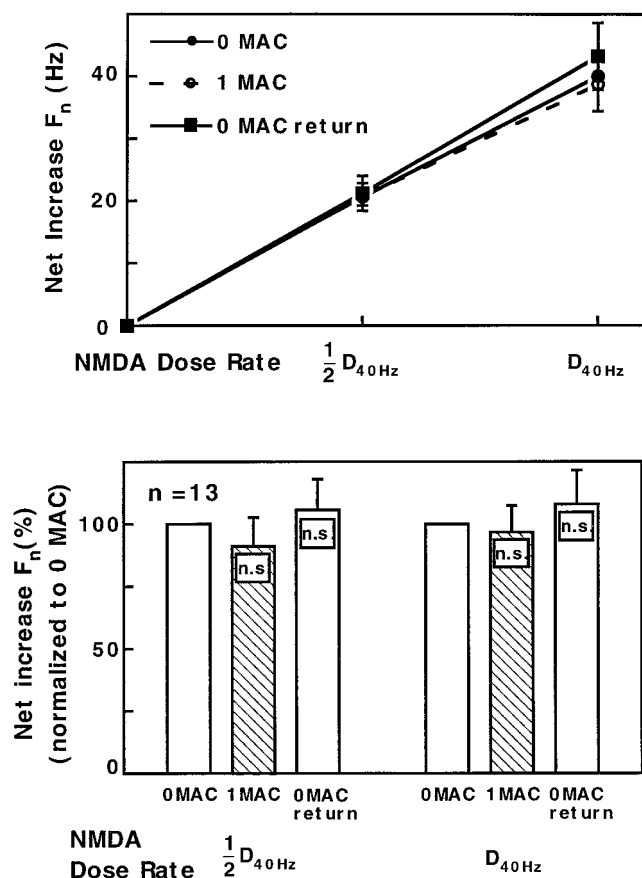


Fig. 6. Pooled summary data of study 2. (Top) Mean values  $\pm$  SD of net increases of peak neuronal discharge frequency ( $F_n$ ) at the  $\frac{1}{2} D_{40\text{Hz}}$  and  $D_{40\text{Hz}}$  N-methyl-D-aspartic acid (NMDA) dose rates for 0–minimum alveolar concentration (MAC), 1 MAC, and 0 MAC end-control for 13 neurons. Halothane, 1 MAC, caused a small reduction in the  $D_{40\text{Hz}}$  net increase from 40 Hz to  $38.7 \pm 15.4$  Hz. (Bottom) Normalized  $\frac{1}{2} D_{40\text{Hz}}$  and  $D_{40\text{Hz}}$  net response data for the same 13 neurons show that the postsynaptic glutamatergic excitatory response was not significantly depressed by the anesthetic.

dogs, in the absence of anesthesia, had a respiratory rate of  $15.4 \pm 8.4$  breaths/min compared with awake, resting animals ( $23.0 \pm 6.3$  breaths/min, our observation from 16 dogs at rest). Decerebrate dogs also show the tachypnea that has been typically associated with halothane at clinical levels. Respiratory rates during 1 MAC halothane were not different from our 1-MAC data obtained in intact animals ( $23.3 \pm 13.2$  vs.  $21.3 \pm 7.1$  breaths/min).<sup>4</sup> This data is different from that of Gautier *et al.*<sup>15</sup> who described a marked reduction of halothane-induced tachypnea in decerebrate compared with intact cats and related this to a suprapontine origin of the halothane tachypnea.

Decerebration removes forebrain and midbrain influences that modify ventilatory responses.<sup>16</sup> It is possible that the elimination of suprapontine excitatory input to the rhythm-generation network allowed apnea to occur at a lower halothane concentration than in neuraxis-intact dogs. This might explain why most of our decer-

brate dogs became apneic at 1.5 MAC halothane even under hypercapnia.

However, for these cVRG E neurons, similar anesthetic-induced reductions in net excitatory drive were observed in both preparations.<sup>5</sup> Furthermore, we found that the basic reflex inputs that we previously described in the intact preparation, including chemodrive<sup>2</sup> and pulmonary vagal inputs, are preserved (unpublished data). Nielsen *et al.*<sup>17</sup> and Mitchell<sup>18</sup> found that respiratory rate, inspiratory duration, and expiratory duration responded to various stimuli, such as bilateral vagotomy, hypercapnia, or hypoxia, but such responses were diminished in decerebrate dogs compared with chloralose-anesthetized dogs.

In the current study, 1 MAC halothane reduced peak phrenic activity (index of tidal volume) to  $52.2 \pm 19.9\%$  of control (0 MAC). This magnitude is very similar to the estimated reduction of tidal volume in awake, spontaneously breathing dogs produced by 1 MAC halothane.<sup>19</sup>

The current studies were performed in a newly developed decerebrate canine preparation that allows the study of volatile anesthetics on brain stem respiratory neuronal activity without the confounding effect of background anesthetic. The studies confirmed that the depressive effect of halothane in cVRG E neurons was mainly the result of a reduction of synaptic excitation with an additional small enhancement of synaptic inhibitory mechanisms. Furthermore, because glutamatergic excitation of postsynaptic neuronal receptors was not depressed by halothane, the main depressant effects of the anesthetic halothane appear to occur at presynaptic sites.

Thus, this new decerebrate model provides additional evidence for the importance of alterations of glutamatergic neuronal transmission by volatile anesthetics in functional neural networks *in vivo*.

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