Effects of Small Concentrations of Volatile Anesthetics on Action Potential Firing of Neocortical Neurons In Vitro

Bernd Antkowiak, Ph.D.,* Charlotte Helfrich-Förster, Ph.D.*

Background: Volatile general anesthetics depress neuronal activity in the mammalian central nervous system and enhance inhibitory Cl⁻ currents flowing across the γ-aminobutyric acid $_A$ (GABA$_A$) receptor–ion channel complex. The extent to which an increase in GABA$_A$-mediated synaptic inhibition contributes to the decrease in neuronal firing must be determined, because many further effects of these agents have been reported on the molecular level.

Methods: The actions of halothane, isoflurane, and enflurane on the firing patterns of single neurons were investigated by extracellular recordings in organotypic slice cultures derived from the rat neocortex.

Results: Volatile anesthetics depressed spontaneous action potential firing of neocortical neurons in a concentration-dependent manner. The estimated median effective concentration (EC$_{50}$) values were about one half the EC$_{50}$ values for general anesthesia. In the presence of the GABA$_A$ antagonist bicuculline (20 μM), the effectiveness of halothane, isoflurane, and enflurane in reducing the discharge rates were diminished by 48–65%, indicating that these drugs act *via* the GABA$_A$ receptor.

Conclusions: Together with recent investigations, our results provide evidence that halothane, isoflurane, and enflurane reduced spontaneous action potential firing of neocortical neurons in cultured brain slices mainly by increasing GABA$_A$-mediated synaptic inhibition. At concentrations, approximately one half the EC$_{50}$ for general anesthesia, volatile anesthetics increased overall GABA$_A$-mediated synaptic inhibition about twofold, thus decreasing spontaneous action potential firing by half. (Key words: Brain; enflurane; γ-aminobutyric acid; glutamate; halothane; isoflurane; neocortex; rat; receptors.)

VOLATILE anesthetics decrease spontaneous and evoked neuronal activity in various parts of the mammalian central nervous system.[1–4] A mechanism that may contribute to neuronal depression concerns the effects reported on the γ-aminobutyric acid $_A$ (GABA$_A$) receptor.[5–16] These actions occur at clinically relevant concentrations and are in accordance with the predictions of the Meyer-Overton rule.[17]

Besides the effects on GABA$_A$ receptors, further molecular effects of volatile anesthetics are believed to be involved in decreasing neuronal activity. They include a depression of glutamatergic synaptic transmission,[18,19] a hyperpolarization of the membrane resting potential,[20,21] a decrease in the input resistance,[20,21] a depression of inward rectification,[21] a shift in the threshold for spike generation,[22,23] a depression of spike after-hyperpolarizations,[20] and a blockade of Ca^{++} currents.[24,25] The question as to which of these actions are really relevant is still controversial.[26–28] For example, Langmoen *et al.*[27] recently argued that, although GABA-induced Cl⁻ currents are potentiated by volatile anesthetics, overall synaptic inhibition might be depressed on the network level, because volatile anesthetics also decrease the firing rates of inhibitory interneurons. However, because of the lack of supporting data, this view is purely speculative. In the current study, we compared the effectiveness of general volatile anesthetics in reducing the mean firing rates of neurons. We used cultured neocortical brain slices in which GABA$_A$-mediated synaptic transmission was either present or not. To attain the latter condition, the specific GABA$_A$ antagonist bicuculline was dissolved in the bathing solution. Because of the molecular mechanism by which volatile anesthetics develop their depressant effects, we expected the following. Should the tested anesthetics act by mechanisms that leave GABA$_A$-mediated synaptic inhibition unaffected, their effectiveness would be similar, regardless of whether bicuculline was present. Otherwise their effectiveness should be considerably reduced in bicuculline-treated preparations.

Materials and Methods

Neocortical Slice Cultures

Neocortical slice cultures were prepared from 1- to 3-day-old Sprague-Dawley rats. Animals of both sexes were anesthetized with enflurane and decapitated. Cortical hemispheres were aseptically removed and stored in ice-cold Gey's balanced salt solution consisting of 1.5 mM $CaCl_2$, 5 mM KCl, 0.22 mM KH_2PO_4, 11 mM $MgCl_2$, 0.3 mM $MgSO_4$, 137 mM NaCl, 0.7 mM $NaHCO_3$, and 33 mM glucose. Hemispheres were glued onto a Teflon block, and sagittal slices 300–400 μm thick were cut with a vibratome. The tissue was stored in a refrigerator at 4°C for 30–45 min. About 20–40 slices were obtained from a single rat. Slices were then transferred onto clean glass coverslips. They were embedded on a plasma clot consisting of 20 μl heparin-treated chicken plasma coagulated by 20 μl of a thrombin solution. The roller tube technique described by Gähwiler was used to culture the tissue.[29] After 1 day in culture, antimitotics (10 μM 5-fluoro-2-deoxyuridine, 10 μM cytosine-b-d-arabino-furanoside, 10 μM uridine) were added. The suspension and the antimitotics were renewed twice a week.

Extracellular Recordings

Slices were viewed with an inverted microscope at low magnification. They were perfused continuously with an artificial cerebrospinal fluid (ACSF) consisting of 120 mM NaCl, 3.3 mM KCl, 1.13 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1.8 mM $CaCl_2$, and 11 mM glucose. The ACSF was bubbled with 95% oxygen and 5% carbon dioxide. Glass electrodes filled with ACSF and resistances of about 5 MΩ were positioned on the surface of the slices. Electrodes were advanced into the tissue until extracellular spikes >100 μV in amplitude were visible and a single unit could be clearly discriminated. The noise amplitude was usually between 20 and 50 μV.

Control of Experimental Temperature

Experiments were done either at 22–24°C or 34–36°C. The recording chamber consisted of a metal frame with a glass bottom. A heating wire was glued onto the metal frame. In cases in which experiments were done at 34–36°C, the frame was heated by passing an appropriate DC current through the heating wire.

Preparation and Application of Test Solutions

Test solutions were prepared by dissolving the anesthetics in their liquid form in the ACSF to yield the desired final concentration. Before dissolving the anesthetics, the ACSF was equilibrated with 95% oxygen and 5% carbon dioxide. A closed, air-free system was used to prevent evaporation of the anesthetics. After vigorously stirring the test solutions for at least half an hour, they were transferred to gas-tight glass syringes. Care was taken to avoid air exposure. To facilitate judgment of whether effects occur in a range of clinically relevant concentrations, and to enable easy comparison between the effects of the different anesthetics, anesthetic levels are also given as multiples of minimum alveolar concentration (MAC). These MAC values refer to the plasma or blood concentrations of volatile anesthetics in mammals, determined at 37°C. We used the EC_{50} values for general anesthesia proposed by Franks and Lieb.[26] Thus we assumed that 1 MAC corresponds to aqueous concentrations of 0.25 mM halothane, 0.32 mM isoflurane, and 0.62 mM enflurane.

In earlier investigations we prepared the test solutions using a different method.[30,31] Briefly, 500 ml ACSF was bubbled at a flow rate of 200 ml/h with a vapor containing the volatile anesthetics. The vapor concentration was delivered by calibrated vaporizers. An equilibrium between the gas and liquid phase was reached after about 30 min.[32] Samples were taken using gas-tight syringes. Continuous bubbling ensured that the anesthetics did not evaporate during this procedure. We calculated anesthetic concentrations in the test solution based on published literature values for aqueous solubility.[33] A bioassay was used to compare the potency of the tested solutions, which were prepared by the different methods described. Because of the low standard deviations of the parameter measured,[30] a difference in anesthetic concentrations in the test solutions of 10–20% could be detected. However, we could not find differences in anesthetic potencies, no matter what method was used to prepare test solutions.[30] Thus we assume the possible error in anesthetic concentrations to be <20%.

Anesthetics were applied *via* bath perfusion using syringe pumps (ZAK, Marktheidenfeld, Germany), which were connected *via* Teflon tubing to the experimental chamber. The flow rate was approximately 1 ml/min. When switching from ACSF to drug-containing solutions, the medium in the experimental chamber was replaced by at least 95% within 2 min. Effects on the spike patterns were stable about 5 min later. This delay may be attributed to the diffusion of the test solutions into the tissue. The time required to observe recovery increased with the concentration tested. With 0.5–2 MAC, full recovery was reached after 12–15 min and

with 2 – 6 MAC after 30 – 60 min. For a single application, stable recording for about 1 h was necessary. The loss of volatile anesthetics from the experimental chamber was negligible.[30]

Data Analysis

Data were either lowpass filtered between 3 and 10 kHz or bandpass filtered (30 Hz to 3 – 10 kHz), as acquired on a personal computer with the digidata 1200 AD/DA interface and pClamp 6 software (Axon Instruments, Foster City, CA). Records were stored simultaneously on a Sony data recorder PC 204A (Racal Elektronik, Bergisch Gladbach, Germany) for further analysis. Extracellularly recorded spikes were counted online or off-line using software event detectors. Average spike rates were measured as the mean of spikes occurring in a period of 180 – 300 s. Interspike interval and burst analysis were performed using the pClamp program package. For statistical analysis, paired Student's *t* tests and analysis of variance were used. Unless otherwise stated, results are given as means ± SE.

Results

Action Potential Firing in Cultured Neocortical Brain Slices at Zero Extracellular Mg^{++}

Cultured brain slices were used to study the actions of volatile anesthetics on neocortical neurons *in vitro*. In contrast to cultures of dissociated neurons, the characteristic layering of neocortical cells is well preserved in this type of preparation, and pyramidal cells differentiate in a similar manner *in vivo*.[34] Extracellular recordings from single neocortical neurons were performed on 562 cells located in layers II – VI either at 22 – 24°C or at 34 – 36°C. At zero extracellular Mg^{++}, spontaneous action potential firing could not be detected during the first week in culture but was commonly observed between 12 – 45 days *in vitro*. Between 15 and 42 days *in vitro*, the mean firing rates remained at approximately constant values. Cultures of the corresponding age were used to analyze the effects of volatile anesthetics. Figure 1 presents a typical recording at 34 – 36°C from a cell in the upper layers, about 150 μm deep in the slice. The neuron fires bursts of action potentials at a rate of about 0.05 Hz (fig. 1A). These bursts appear overlayed with a transient change in the field potential (fig. 1B). At the beginning of each burst, the cell discharges at a high rate (about 400 Hz) and the amplitudes of action potentials transiently decrease

(fig. 1C). The firing rates at the end of the bursts range from 20 – 60 Hz. In the same recording, the width of extracellularly recorded spikes is about 1 ms (fig. 1D). Similar firing patterns were observed in about 70% of the slices. In the remaining cases, a mixture of burst firing and single spiking was seen (see fig. 5, for example).

Distributions of the mean discharge rates, observed in the absence of drugs, are shown in figures 1E and F. Decreasing the temperature from 34 – 36°C to 22 – 24°C caused only a small decrease in the mean spike rates. However, a change of temperature altered the discharge patterns. At the lower temperature, the burst frequency was higher (at 23°C, 0.27 ± 0.025 Hz; at 35°C, 0.15 ± 0.014 Hz), whereas the discharge rates within the bursts decreased by about half (compare figures 2, 4, and 5).

Spontaneous excitatory synaptic events were recorded in neocortical slices after a few days in culture, indicating that synaptic connections had developed between the cells. If the perfusion media contained about 1 mM Mg^{++}, spontaneous synaptic excitation was too low to cause action potential firing at considerable rates. In contrast, firing patterns, such as those shown in figure 1, commonly developed after removing extracellular Mg^{++} ions from the bathing solution, probably by removing the voltage-dependent Mg^{++} block of N-methyl-D-aspartate receptors. The involvement of these receptors in causing action potential firing was directly demonstrated by applying the glutamate antagonist 2-amino-5-phosphonopentanoic acid (AP-5). As shown in figure 2, AP-5 decreased the discharge rates by lengthening interburst intervals and increasing the interspike intervals within the bursts. To facilitate automatic spike counting in this and subsequent recordings, the changes in the field potential were removed by highpass filtering the voltage traces at 30 Hz, leaving the fast sodium spikes. Half-maximal depression of the firing rates was observed with 3.4 μM AP-5, as interpolated from the concentration-response curve shown in figure 3.

Effects of Volatile Anesthetics on the Discharge Patterns

Typical examples of the effects of halothane at 22 – 24°C and isoflurane at 34 – 36°C on the discharge patterns of neocortical neurons are shown in figures 4 and 5. Bath application of 0.375 mM halothane (corresponding to 1.5 MAC) largely depressed action potential firing. The effects on the firing patterns were very similar to those observed during AP-5 treatment. At high concentrations (>1 MAC), halothane increased interburst
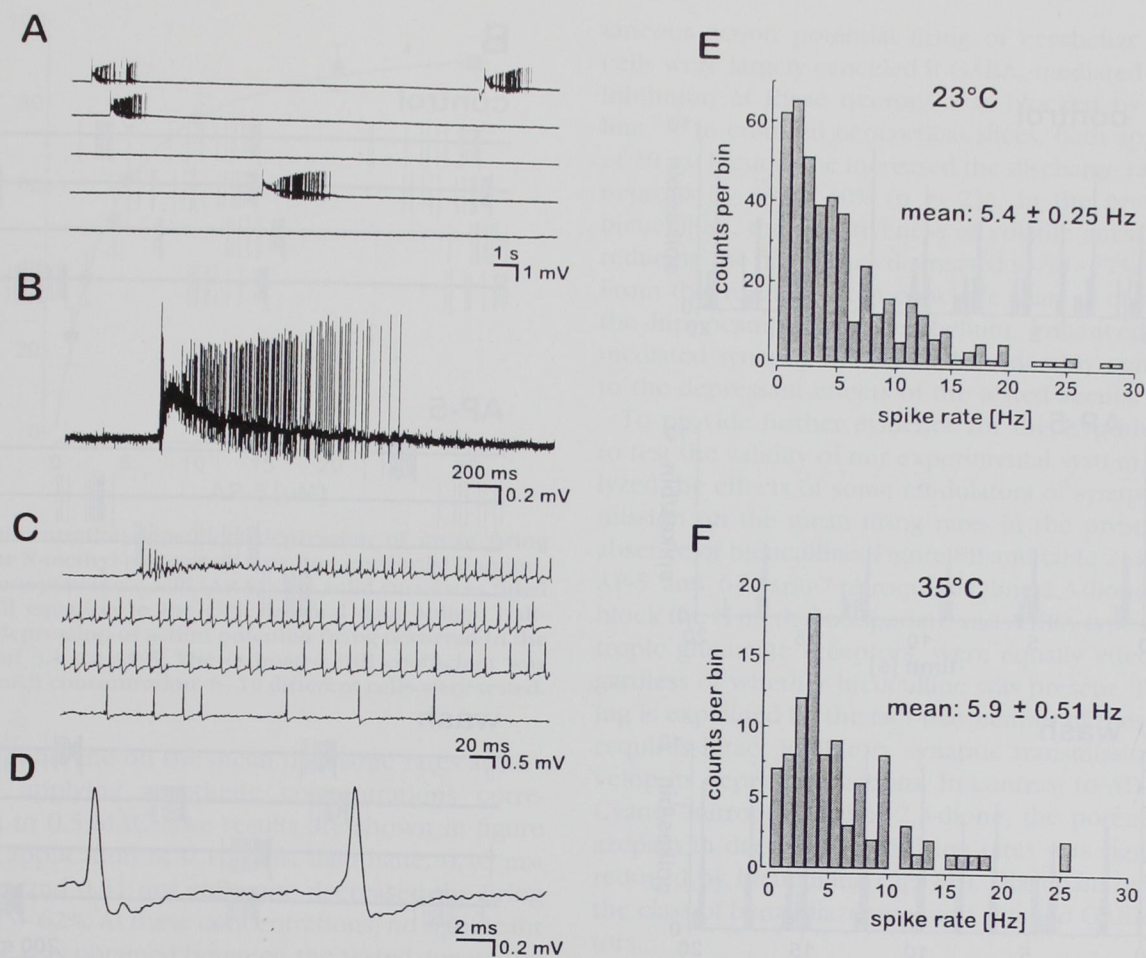
Fig. 1. Spontaneous action potential firing of neurons in cultured neocortical brain slices at zero extracellular Mg^{++}. Examples of typical extracellular recordings made at 34–36°C and lowpass filtered at 4 kHz. (*A*) Spike patterns at a low time resolution. Action potentials were grouped into bursts that occurred at a frequency of about 0.05 Hz. (*B*) During the bursts, a transient shift in the field potential appeared overlayed with fast action potentials. (*C*) A burst of action potentials at higher time resolution. At the onset of the burst, the cell fired five to seven spikes with decreasing amplitudes. After about 80 ms, spike amplitudes recovered and the cell fired at a rate of 20–60 Hz. (*D*) Single action potentials at a higher time resolution. (*E*) Distribution of average spike rates at 22–24°C. (*F*) Distribution of average spike rates at 34–36°C. The mean discharge rates did not differ significantly ($P < 0.05$) between the different temperatures.

durations and simultaneously lengthened the interspike intervals within the bursts, whereas at lower concentrations the decrease in the average discharge rate was mainly caused by a decrease in the burst frequency. In figure 5, 0.16 and 0.32 mM isoflurane (corresponding to 0.5 and 1 MAC) were applied to another neuron. As in the case of halothane, isoflurane increased the interburst durations and decreased the firing rate within the bursts. As the concentration of isoflurane was increased to 0.62 mM (corresponding to 2 MAC), spontaneous firing was completely abolished (data not shown). The results presented in figure 6 summarize the effects of isoflurane on the mean discharge rates,

the discharge rate within bursts, the number of spikes per burst, and the interburst durations. In figure 6A, the effects of isoflurane on the average firing rates and the average number of action potentials per burst are compared. The reduction of the mean discharge rate was considerably larger than the decrease in the number of action potentials per burst. Thus the anesthetic-induced lengthening of the interburst intervals shown in figure 6B was the most important effect in causing the depression of the average firing rates. The decrease in the number of action potentials per burst resulted from a decrease in the discharge rate during burst firing, but also from a shortening of the bursts (fig. 6C).
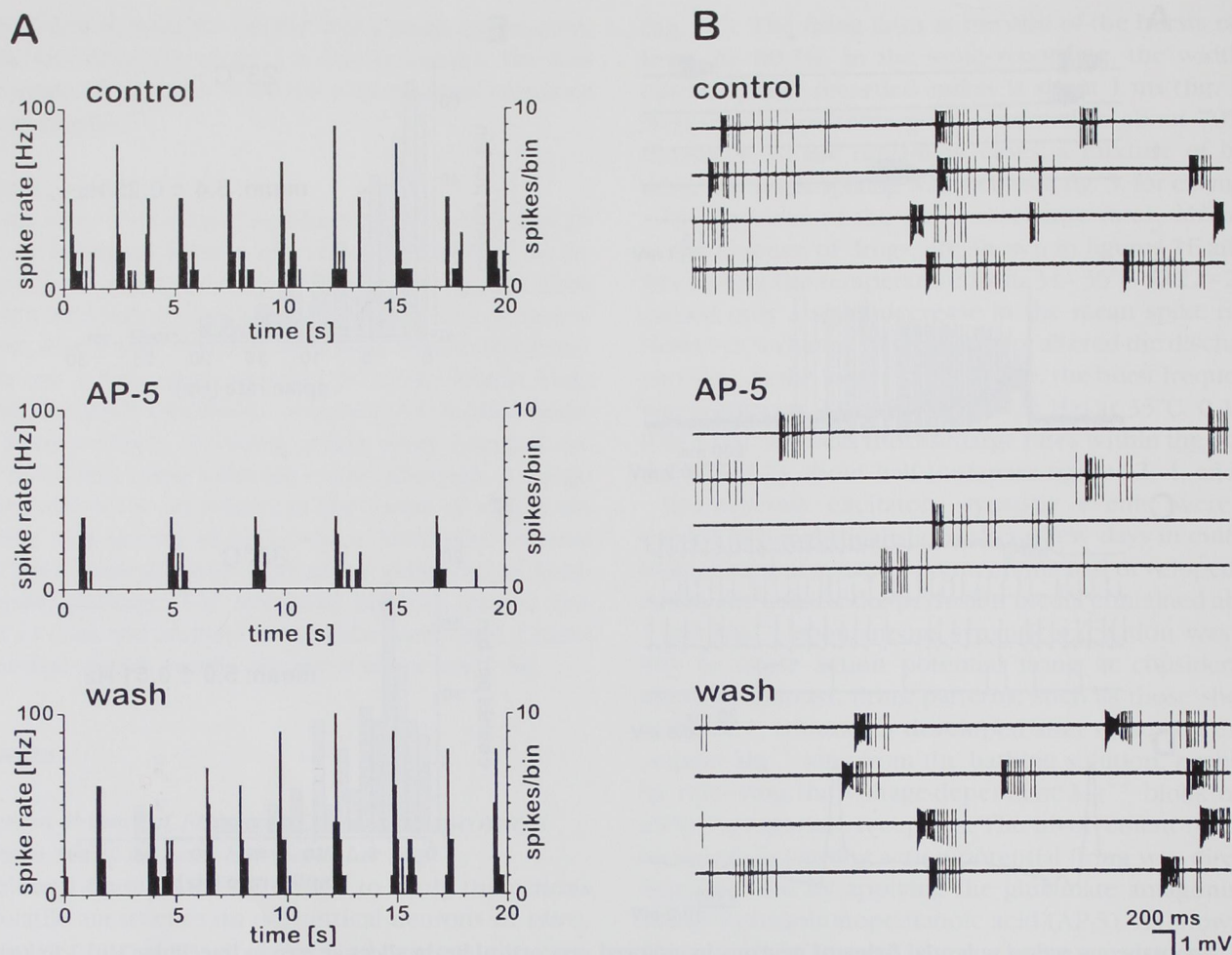
Fig. 2. Effects of 5 μM 2-amino-5-phosphonopentanoic acid (AP-5) on spontaneous action potential firing of a neocortical neuron. The recording was carried out at room temperature. (*A*) Spike rates and (*B*) corresponding original recordings. Mean firing rates were 7.83 Hz before (control), 3.18 Hz during (AP-5), and 9.59 Hz after (wash) treatment.

Effects of Volatile Anesthetics on the Average Discharge Rates

Figure 7 presents the concentration-dependent effects of volatile anesthetics on the average discharge rates of neocortical neurons as observed at 22–24°C and at 34–36°C. Aqueous concentrations are also indicated as MAC values. They were derived from the EC_{50} values for general anesthesia at 37°C proposed by Franks and Lieb[26] (0.25 mM halothane, 0.32 mM isoflurane, 0.62 mM enflurane). These concentrations were considered equivalent to 1 MAC. In figure 7A, average spike rates monitored at various anesthetic concentrations are shown. The depressant effects were quantified by comparing the firing rates of the same cell before and during the treatment. The resulting concentration–response relations in figure 7B were fitted by Hill equations. The insets indicate the 95% confidence limits of the curves, which were adapted to the data points calculated from the experiments conducted at 34–36°C. Table 1 summarizes the EC_{50} values and Hill coefficients derived from the concentration–response fits. With all anesthetics and at both temperature ranges, half-maximal depression of firing rates occurred at concentrations 0.3–0.7 times the EC_{50} value for general anesthesia.

Furthermore, we wanted to determine whether the actions of volatile anesthetics in neocortical slice cultures are in accordance with the Meyer-Overton rule. The rule predicts that the depressant effects should not differ if the compounds are applied at the same MAC values. We compared the effects of halothane, isoflur-
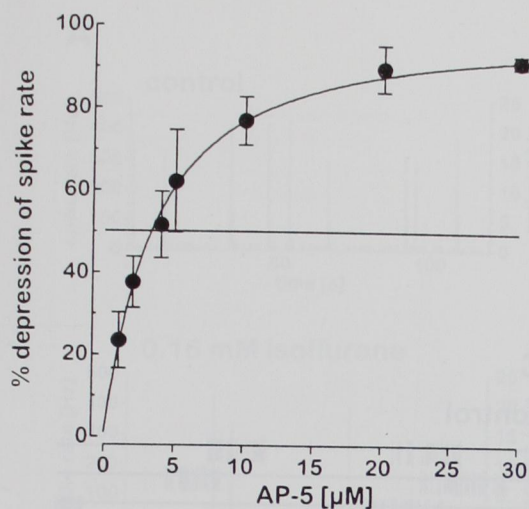
Fig. 3. Concentration-dependent depression of mean firing rates by the N-methyl-D-aspartate receptor antagonist 2-amino-5-phosphonopentanoic acid (AP-5). The solid curve was fitted with a Hill equation to the experimental data points. Half-maximal depression of action potential firing occurred in the presence of 3.4 μM AP-5. The estimated Hill coefficient was 1.07. For each concentration, 6–10 different cells were tested.

ane, and enflurane on the mean discharge rates at 34–36°C by applying anesthetic concentrations corresponding to 0.5 MAC. The results are shown in figure 8A. Bath application of 0.125 mM halothane, 0.16 mM isoflurane, and 0.31 mM enflurane decreased the firing rates by 45–62%. At these concentrations, no significant difference was obtained between the tested agents (by analysis of variance, $P < 0.05$). Thus we were not in a position to reject the hypothesis that the actions of the three volatile anesthetics follow the Meyer-Overton rule.

Another interesting question concerns the temperature dependence of anesthetic potencies. If plasma or blood concentrations, but not alveolar concentrations, are considered, the MAC requirement *in vivo* decreases by 20–25% if temperature is decreased from 36°C to 23°C.[35] Considering that the MAC changes with the experimental temperature, anesthetic concentrations applied at 23°C were reduced to 80% of those tested at 36°C. The results in figure 8A show that, also with regard to the temperature dependence, our results were not at variance with the expectations derived from *in vivo* studies.

Effects of Volatile Anesthetics in the Presence of the GABA_A Antagonist Bicuculline

The depressant effects of volatile anesthetics on stimulus-evoked firing of hippocampal neurons and on spontaneous action potential firing of cerebellar Purkinje cells were largely canceled if GABA$_A$-mediated synaptic inhibition of these neurons was blocked by bicuculline.[7,30] In cultured neocortical slices, bath application of 20 μM bicuculline increased the discharge rate of the neurons by $39 \pm 30\%$ (n = 23). In the presence of bicuculline, the effectiveness of volatile anesthetics in reducing the firing rates decreased to 58–77% (fig. 8B). From this result we can conclude that, as observed in the hippocampus and cerebellum, enhanced GABA$_A$-mediated synaptic inhibition considerably contributed to the depressant effects of the tested agents.

To provide further evidence for this hypothesis and to test the validity of our experimental system, we analyzed the effects of some modulators of synaptic transmission on the mean firing rates in the presence and absence of bicuculline. Figure 8B and table 2 show that AP-5 and 6-Cyano-7-nitroquinoxaline-2,3-dione, which block the N-methyl-D-aspartate and AMPA types of ionotropic glutamate receptors, were equally effective, regardless of whether bicuculline was present. This finding is explained by the fact that neither of these agents requires intact inhibitory synaptic transmission to develop its depressant actions. In contrast to AP-5 and 6-Cyano-7-nitroquinoxaline-2,3-dione, the potency of diazepam in decreasing the firing rates was significantly reduced by bicuculline (fig. 8B). Diazepam belongs to the class of benzodiazepines and acts *via* GABA$_A$ receptors.

Discussion

Relation between Anesthetic Actions on the Molecular and Network Levels

The mechanisms by which volatile anesthetics reduce neuronal activity in the mammalian central nervous system are still a matter of controversy.[26-28] In the present work, we have compared the effects of halothane, isoflurane, and enflurane on the spontaneous action potential firing of neocortical neurons *in vitro* in the absence and presence of the competitive GABA$_A$ antagonist bicuculline. Similar to what has already been observed in hippocampal and cerebellar slices,[7,30] the effectiveness of these anesthetics in depressing the mean firing rates decreased considerably if the binding of the neurotransmitter GABA to the GABA$_A$ receptor was reduced by the bicuculline. This result indicates that volatile anesthetics develop their depressant actions on the network level *via* the GABA$_A$ channel by
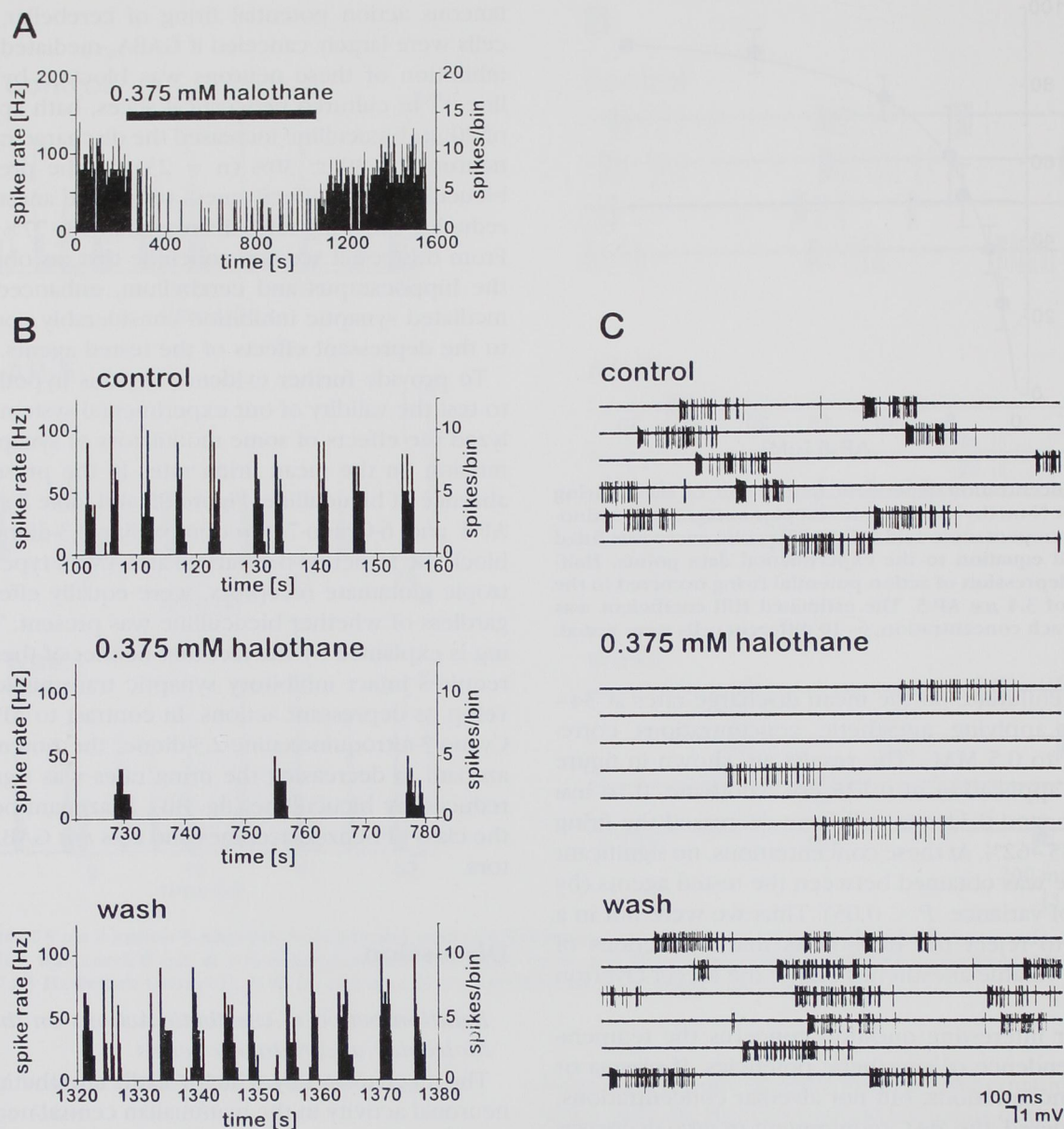
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Fig. 4. Effects of 0.375 mM (1.5 minimum alveolar concentration) halothane on spontaneous action potential firing of a neocortical neuron. (*A*) Spike rates while halothane was applied. Action potentials were binned at 100 ms-intervals. (*B, C*) The spike rates and discharge patterns of the same cell before, during, and after halothane administration. Mean spike rates were 7.87 Hz (control), 1.56 Hz (0.375 mM halothane), and 7.51 Hz (wash). The temperature was 22–24°C.

enhancing GABA-induced Cl⁻ currents. In contrast to volatile anesthetics, the effectiveness of AP-5 and 6-Cyano-7-nitroquinoxaline-2,3-dione in reducing the firing rates were not decreased by bicuculline (fig. 8B).

Even in the presence of 20 μM bicuculline, volatile anesthetics decreased action potential firing of neocortical cells by 5–20% (fig. 8A). These residual effects may

indicate that the GABA_A receptor is not the exclusive target involved in the depression of firing rates. Alternatively, they could arise from an incomplete blockade of GABA_A receptors. Based on our data, we cannot distinguish between these possibilities. The discovery that the effects of diazepam, which acts more specifically on GABA_A receptors than volatile anesthetics do, were
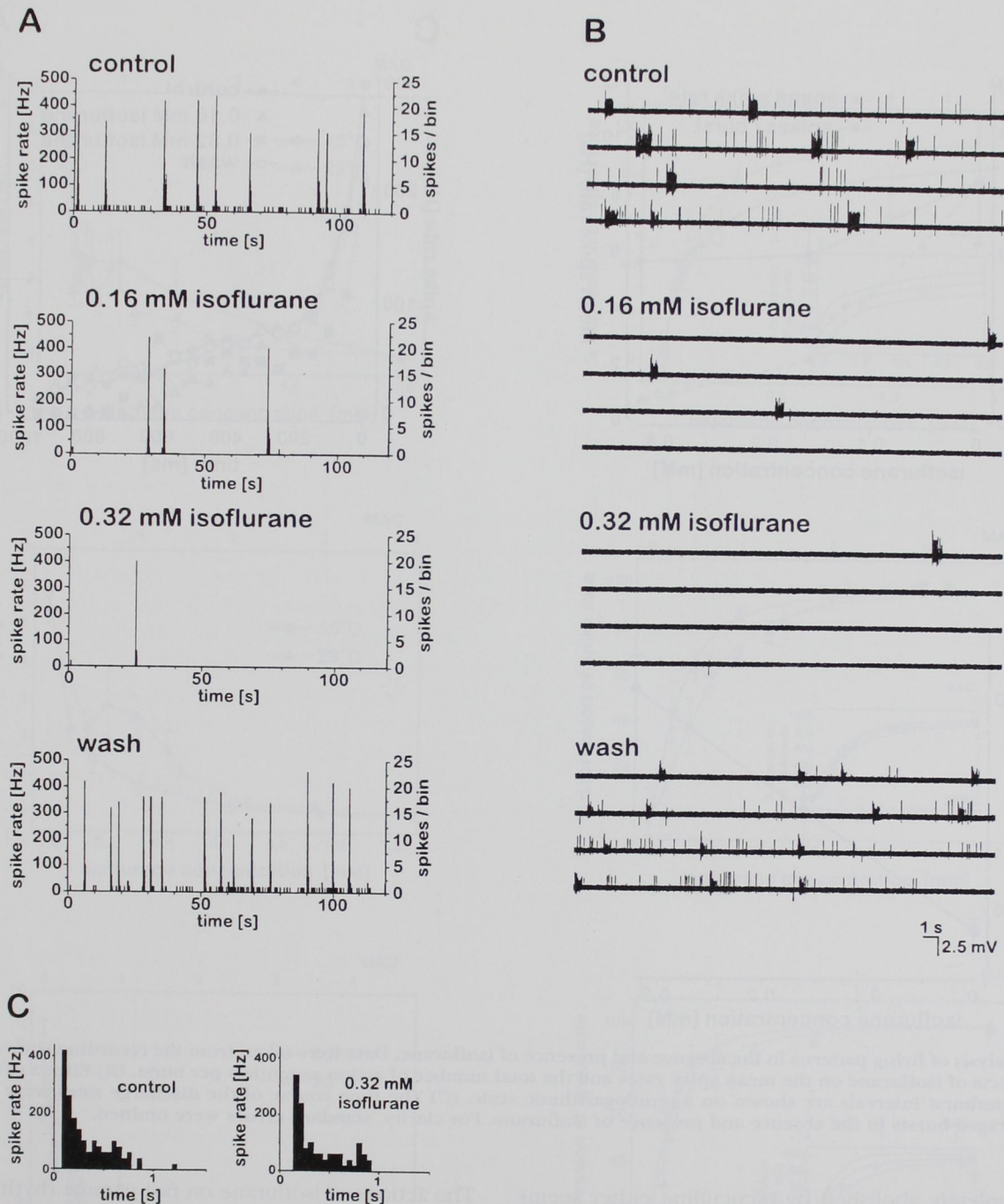
Fig. 5. Concentration-dependent actions of isoflurane on the discharge patterns of a neuron. The anesthetic was applied at 0.16 mM (0.5 minimum alveolar concentration [MAC]) and 0.32 mM (1 MAC). (*A*, *B*) Discharge rates and the corresponding original recordings in the absence and presence of the anesthetic. The temperature was 34–36°C. Burst rates were 0.083 Hz (control), 0.025 Hz (0.16 mM), 0.0083 Hz (0.32 mM) and 0.12 Hz (wash). Spikes were binned at 50 ms-intervals. The corresponding average discharge rates were 6.43 Hz (control), 2.03 Hz (0.16 mM), 0.61 Hz (0.32 mM), and 8.6 Hz (wash). (*C*) Comparison of the discharge rates during single bursts before and during isoflurane treatment.
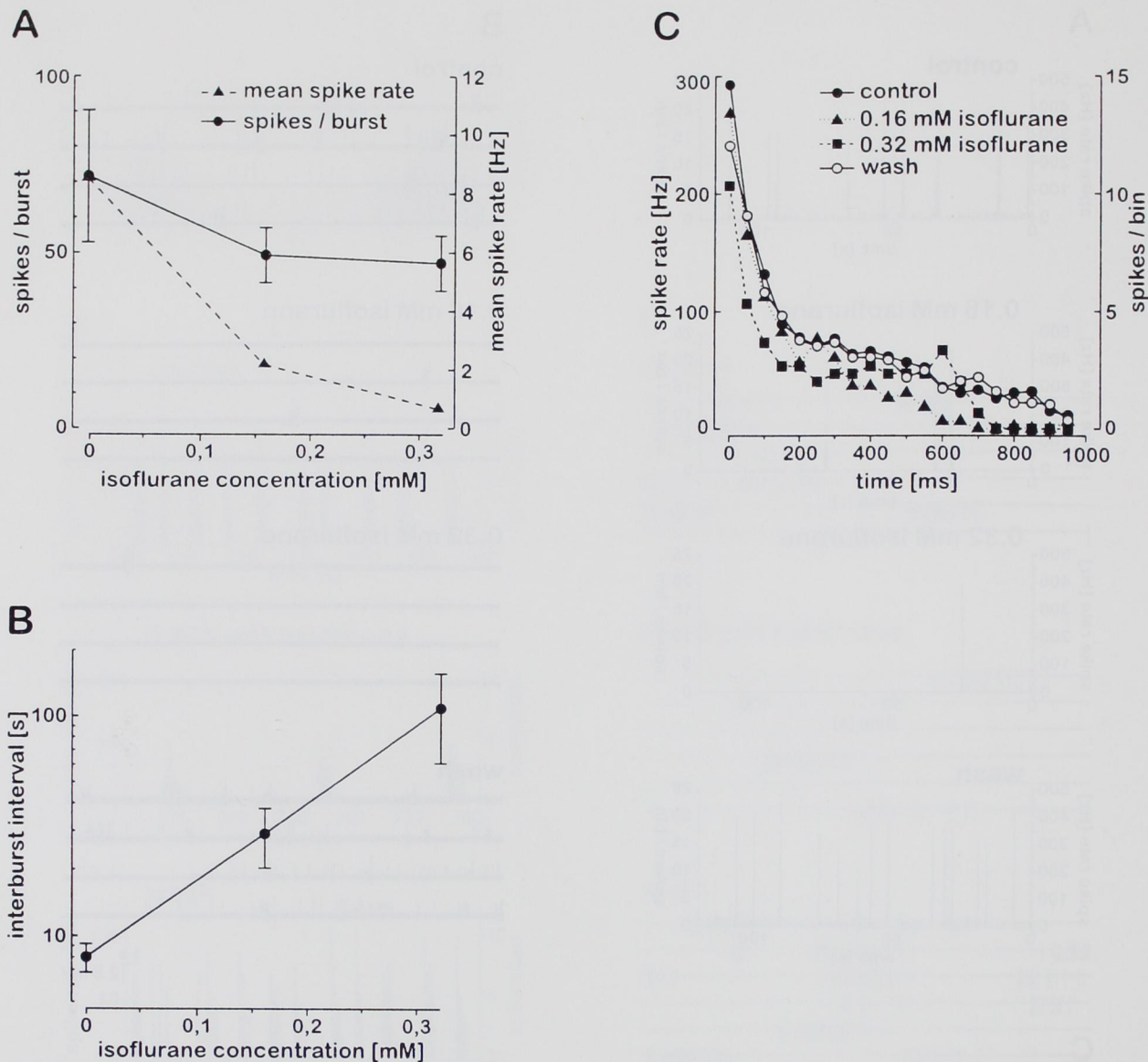
A

B

C

Fig. 6. Analysis of firing patterns in the absence and presence of isoflurane. Data were taken from the recording shown in figure 5. (*A*) Effects of isoflurane on the mean spike rates and the total number of action potentials per burst. (*B*) Effects of isoflurane on the interburst intervals are shown on a semilogarithmic scale. (*C*) The time course of the discharge rates were calculated from averaged bursts in the absence and presence of isoflurane. For clarity, standard errors were omitted.

not completely abolished by bicuculline either seems to argue for the latter possibility. However, even for benzodiazepines some side effects have been reported.[36]

The conclusion that small concentrations of volatile anesthetics significantly increase GABA$_A$-mediated synaptic inhibition recently was confirmed directly when investigators recorded synaptic events from voltage-clamped pyramidal cells in neocortical brain slices.[37]

The actions of isoflurane on fast gamma rhythms in the local field potential reflecting the synchronous synaptic activity of neocortical neurons were analyzed in the same study. Neocortical high-frequency oscillations (about 40 Hz) have become considerably more interesting ever since a correlation between the occurrence of these rhythms and the state of conscious awareness was suggested.[38] Whittington *et al.*[39] provided evidence that these oscillations are generated by GABAergic interneu-
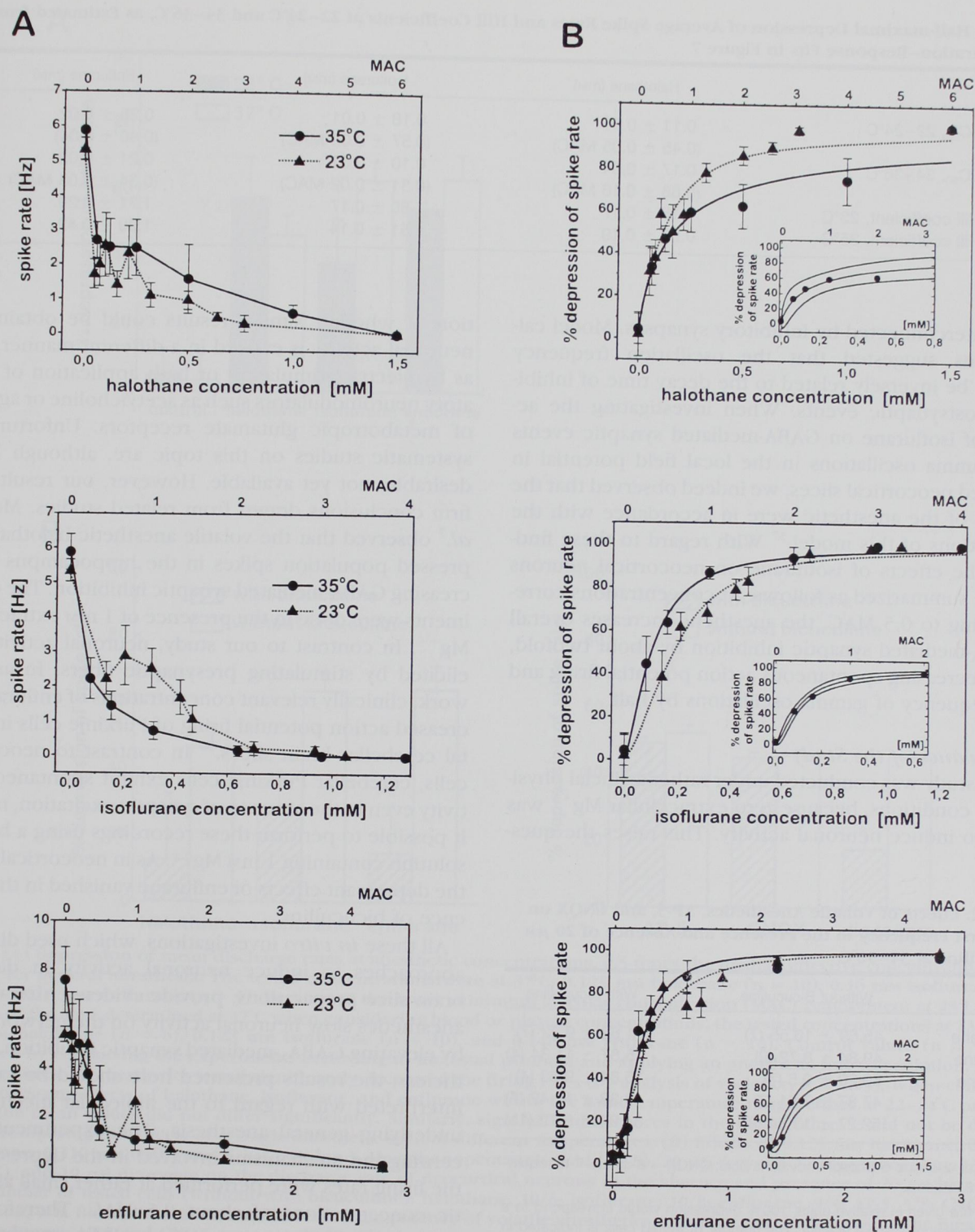
Fig. 7. Concentration–response relations of anesthetic-induced depression of mean spike rates. For each concentration, the mean value and standard error were calculated from 7–15 tested cells. (*A*) Averaged spike rates monitored at 22–24°C (*triangles, dotted line*) and 34–36°C (*circles, solid line*). (*B*) The effects of volatile anesthetics were calculated by comparing action potential discharge rates before and during treatment. The curves were fitted with Hill equations to the data points. Table 1 shows the median effective concentration (EC_{50}) values and Hill coefficients. The insets indicate the adapted curves (34–36°C) and the 95% confidence limits close to the estimated EC_{50} values.

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Table 1. Half-maximal Depression of Average Spike Rates and Hill Coefficients at 22–24°C and 34–36°C, as Estimated from the Concentration–Response Fits in Figure 7

	Halothane (mM)	Isoflurane (mM)	Enflurane (mM)
EC_{50}, 22–24°C	0.11 ± 0.01 (0.45 ± 0.05 MAC)	0.18 ± 0.01 (0.57 ± 0.03 MAC)	0.25 ± 0.02 (0.40 ± 0.04)
EC_{50}, 34–36°C	0.17 ± 0.04 (0.68 ± 0.16 MAC)	0.10 ± 0.01 (0.31 ± 0.02 MAC)	0.21 ± 0.02 (0.34 ± 0.03 MAC)
Hill coefficient, 23°C	1.15 ± 0.17	1.80 ± 0.17	1.71 ± 0.29
Hill coefficient, 35°C	0.80 ± 0.19	1.51 ± 0.14	1.99 ± 0.42

rons interconnected by inhibitory synapses. Model calculations suggested that the oscillation frequency should be inversely related to the decay time of inhibitory postsynaptic events. When investigating the actions of isoflurane on GABA-mediated synaptic events and gamma oscillations in the local field potential in cultured neocortical slices, we indeed observed that the effects of the anesthetic were in accordance with the predictions of this model.[37] With regard to these findings, the effects of isoflurane on neocortical neurons can be summarized as follows: At concentrations corresponding to 0.5 MAC, the anesthetic increases overall $GABA_A$-mediated synaptic inhibition by about twofold, thus decreasing spontaneous action potential firing and the frequency of gamma oscillations by half.

Relevance of the Study

Our study was conducted under rather artificial physiologic conditions, because zero extracellular Mg^{++} was used to induce neuronal activity. This raises the question of whether similar results could be obtained, if neuronal activity is evoked in a different manner, such as by electric stimulation or bath application of excitatory neuromodulators such as acetylcholine or agonists of metabotropic glutamate receptors. Unfortunately, systematic studies on this topic are, although highly desirable, not yet available. However, our results confirm conclusions drawn from related studies. Mody *et al.*[7] observed that the volatile anesthetic halothane depressed population spikes in the hippocampus by increasing GABA-mediated synaptic inhibition. The experiments were done in the presence of 1 mM extracellular Mg^{++}. In contrast to our study, neuronal activity was elicited by stimulating presynaptic fibers. In another work, clinically relevant concentrations of enflurane decreased action potential firing of Purkinje cells in sagittal cerebellar brain slices.[30] In contrast to neocortical cells, cerebellar Purkinje cells exhibit spontaneous activity even in the absence of synaptic excitation, making it possible to perform these recordings using a bathing solution containing 1 mM Mg^{++}. As in neocortical slices, the depressant effects of enflurane vanished in the presence of bicuculline.

All these *in vitro* investigations, which used different approaches to induce neuronal activity in different brain slice preparations, provide evidence that volatile anesthetics slow neuronal activity on the network level by elevating $GABA_A$-mediated synaptic inhibition. Nonetheless, the results presented here should be carefully interpreted with regard to the molecular mechanisms underlying general anesthesia. The experiments concerning the mechanisms involved in the depression of the firing rates were performed at rather small anesthetic concentrations of about 0.5 MAC. Therefore the tested anesthetics might act in a different manner at concentrations corresponding to 1 or 2 MAC, which are necessary to achieve surgical anesthesia. It seems possible, if not likely, that, in addition to the effects on

Table 2. Effects of Volatile Anesthetics, AP-5, and CNQX on the Burst Frequency in the Presence and Absence of 20 μM Bicuculline at 34–36°C

	Without Bicuculline	With Bicuculline
Halothane	45.69 ± 16.55 (7)	1.62 ± 9.32 (7)
Isoflurane	49.84 ± 8.72 (9)	5.87 ± 11.34 (4)
Enflurane	60.20 ± 9.5 (7)	−15.84 ± 9.63 (4)
AP-5	47.87 ± 7.91 (7)	49.38 ± 11.42 (7)
CNQX	52.27 ± 13.54 (8)	34.92 ± 5.72 (11)

AP-5 = DL-2-amino-5-phosphonovaleric acid; CNQX = 6-cyano-7-nitroquinoxaline-2,3-dione.

Results are given in relative units; 100% depression would correspond to a total depression of spike activity. The number of tested cells is indicated in parentheses. The depressant action of AP-5 (5 μM) and CNQX (20 μM) was not significantly affected by the $GABA_A$-antagonist, whereas bicuculline treatment significantly decreased the potencies of halothane (0.125 mM), isoflurane (0.16 mM), and enflurane (0.31 mM) in reducing the burst frequencies ($P < 0.05$, ANOVA).
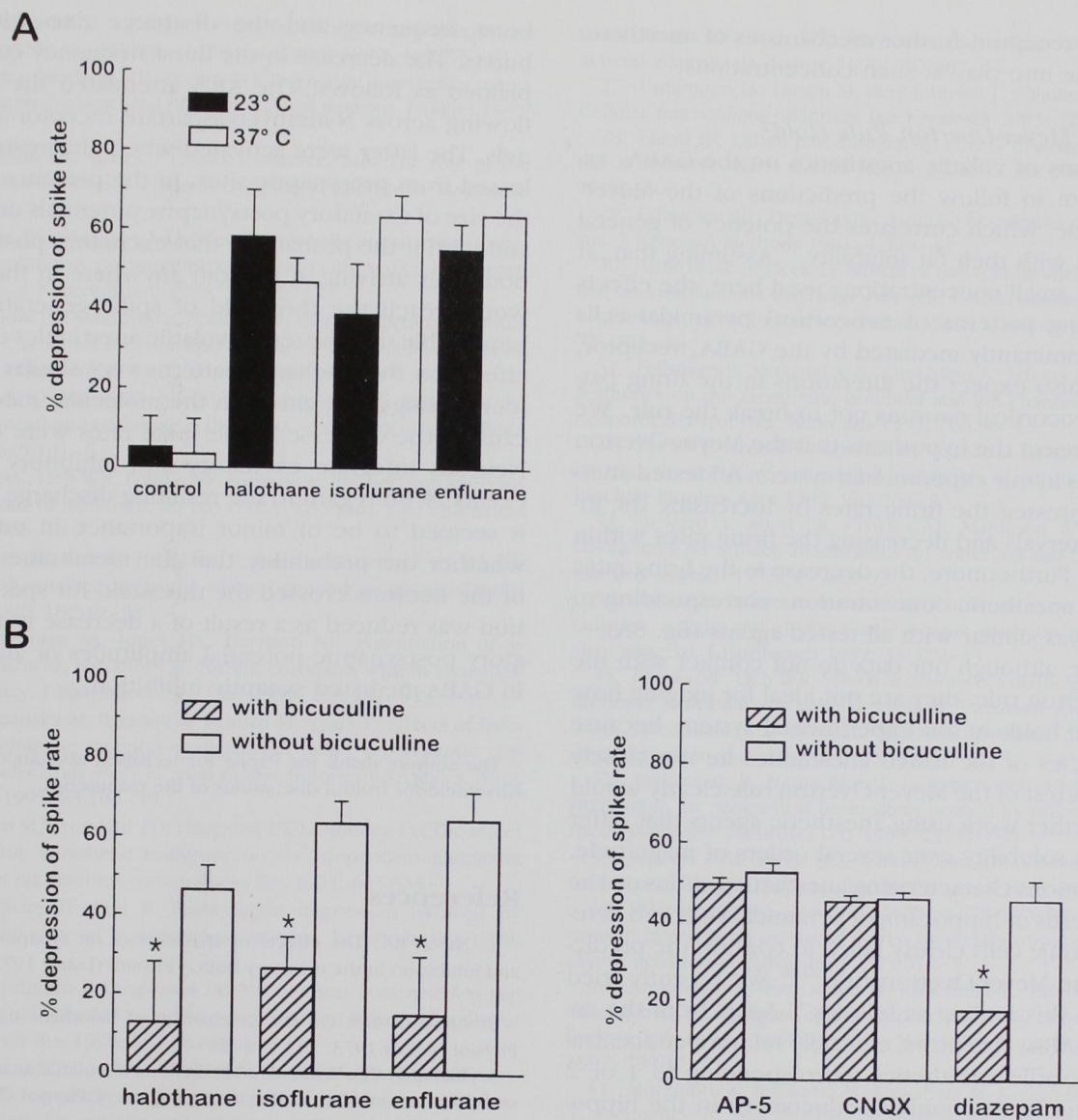
Fig. 8. (*A*) Depression of mean discharge rates at anesthetic concentrations, 0.5 times the median effective concentration (EC_{50}) values for general anesthesia. The tested concentrations were at 37°C: 0.125 mM halothane (n = 10), 0.16 mM isoflurane (n = 10), and 0.31 mM enflurane (n = 9). Because of the lower minimum alveolar concentration (MAC) requirement at 23°C, which is 80% of the MAC determined at 37°C when considering blood or plasma concentrations, the tested concentrations at 23°C were 0.1 mM halothane (n = 6), 0.128 mM isoflurane (n = 10), and 0.248 mM enflurane (n = 10). Control values (n = 5) were obtained by conducting the experiments according to the usual protocol and applying an anesthetic-free test solution. At both temperatures, all anesthetics tested significantly decreased the firing rates (by analysis of variance, $P < 0.05$). When comparing the depressant effects of halothane, isoflurane, and enflurane within the same temperature range, either at 22–24°C or at 35–37°C, the mean values did not differ significantly. Similarly, significant differences in the mean values could not be detected when comparing the effects of a single anesthetic seen at different temperatures. (*B*) Effects of 0.125 mM halothane, 0.16 mM isoflurane, 0.310 mM enflurane, 5 μM 2-amino-5-phosphonopentanoic acid (AP-5), 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and 10 μM diazepam on the discharge rate of neocortical neurons in the absence and presence of 20 μM bicuculline. The number of tested cells (without/with bicuculline): halothane, 10/6; isoflurane, 10/8; enflurane, 9/4; AP-5, 5/8; CNQX, 8/9; diazepam, 9/8. Bicuculline treatment decreased the potency of volatile anesthetics and diazepam in depressing the mean spike rates, whereas AP-5 and CNQX were similarly effective, regardless of whether bicuculline was present. All experiments were performed at 34–36°C. Statistically significant effects are indicated by the asterisks (by analysis of variance, $P < 0.05$).

the GABA_A receptor, further mechanisms of anesthetic action come into play at such concentrations.

Does the Meyer-Overton Rule Hold?

The actions of volatile anesthetics on the GABA_A receptor seem to follow the predictions of the Meyer-Overton rule, which correlates the potency of general anesthetics with their fat solubility.[17] Assuming that, at least at the small concentrations used here, the effects on the firing patterns of neocortical pyramidal cells were predominantly mediated by the GABA_A receptor, we might also expect the alterations in the firing patterns of neocortical neurons not to break the rule. We could not reject the hypothesis that the Meyer-Overton rule applies in our experimental system. All tested anesthetics depressed the firing rates by increasing the interburst intervals and decreasing the firing rates within the bursts. Furthermore, the decrease in the firing rates caused by anesthetic concentrations corresponding to 0.5 MAC was similar with all tested agents (fig. 8A).

However, although our data do not conflict with the Meyer-Overton rule, they are not ideal for judging how far the rule holds in our experimental system, because the potencies of the tested anesthetics lie too closely together. A test of the Meyer-Overton rule clearly would involve further work using anesthetic agents that differ in their fat solubility over several orders of magnitude.

Investigations characterizing anesthetic actions on the firing patterns of hippocampal pyramidal cells and cerebellar Purkinje cells clearly failed to confirm the predictions of the Meyer-Overton rule.[22,40] We recently tried to explain this finding as follows.[40] Apart from the actions on GABA_A receptors, clinically relevant concentrations of volatile anesthetics (corresponding to 1 or 2 MAC) affect further ionic conductances in the hippocampus and cerebellum. Thus anesthetic actions, which are in accordance with the rule, are frequently masked by side effects in these systems. We speculate that these side effects did not occur at the lower concentrations (corresponding to 0.5 MAC) that we tested.

Effects of Volatile Anesthetics on Firing Patterns

Burst firing of neocortical neurons in Mg^{++}-free perfusion media has been investigated in great detail.[41-43] Maeda *et al.*[43] showed that spontaneous bursts were initiated at random locations in the tissue, if excitatory postsynaptic potentials arriving coincidentally shift the membrane potential across the threshold for spike generation. As shown in figures 3 and 4, bath application of the N-methyl-D-aspartate antagonist AP-5 reduced the burst frequency and the discharge rate within the bursts. The decrease in the burst frequency can be explained as follows. The AP-5 attenuated the currents flowing across N-methyl-D-aspartate receptor-ion channels. The latter were activated when glutamate was released from presynaptic sites. In the presence of AP-5, the size of excitatory postsynaptic potentials decreased, and with it the probability that excitatory postsynaptic potentials arriving at random anywhere in the culture would reach the threshold of spike generation. It is remarkable that the tested volatile anesthetics exhibited effects on the discharge patterns very similar to those found using AP-5, although the molecular mechanisms causing the decrease in the spike rates were different. Neurons integrate excitatory and inhibitory synaptic events. With regard to the resulting discharge patterns, it seemed to be of minor importance in our system whether the probability that the membrane potential of the neurons crossed the threshold for spike generation was reduced as a result of a decrease in the excitatory postsynaptic potential amplitudes or an increase in GABA-mediated synaptic inhibition.

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