A Major Role For Tonic GABA\(_A\) Conductances In Anesthetic Suppression Of Intrinsic Neuronal Excitability

Mark C. Bieda and M. Bruce MacIver

Neuroscience Program and Department of Anesthesia, Stanford University, Stanford, California 94305

Contact information (M. Bruce MacIver):
Address: SUMC 288 MC5117
Stanford, CA 94305
Email: maciver@stanford.edu
Phone: (650) 725-5872
FAX: (650) 725-8052
Abstract

Anesthetics appear to produce neurodepression by altering synaptic transmission and/or intrinsic neuronal excitability. Propofol, a widely used anesthetic, has proposed effects on many targets, ranging from sodium channels to GABA\(_A\) inhibition. We examined effects of propofol on the intrinsic excitability of hippocampal CA1 neurons (primarily interneurons) recorded from adult rat brain slices. Propofol strongly depressed action potential production induced by direct current injection, synaptic stimulation, or high-potassium solutions. Propofol-induced depression of intrinsic excitability was completely reversed by bicuculline and picrotoxin, but was strychnine-insensitive, implicating GABA\(_A\) but not glycine receptors. Propofol strongly enhanced inhibitory postsynaptic currents (IPSCs) and induced a tonic GABA\(_A\)-mediated current. We pharmacologically differentiated tonic and phasic (synaptic) GABA\(_A\)-mediated inhibition using the GABA\(_A\) receptor antagonist SR95531 (gabazine). Gabazine (20 µM) completely blocked both evoked and spontaneous IPSCs, but failed to block the propofol-induced depression of intrinsic excitability, implicating tonic, but not phasic, GABA\(_A\) inhibition. Glutamatergic synaptic responses were not altered by propofol (up to 30 µM). Similar results were found in both interneurons and pyramidal cells and with the chemically unrelated anesthetic thiopental. These results suggest that suppression of CA1 neuron intrinsic excitability, by these anesthetics, is largely due to activation of tonic GABA\(_A\) conductances; although other sites of action may play important roles in affecting synaptic transmission, which also can produce strong neurodepression. We propose that, for some anesthetics, suppression of intrinsic excitability, mediated by tonic GABA\(_A\) conductances, operates in conjunction with effects on synaptic transmission, mediated by other mechanisms, to depress hippocampal function during anesthesia.

Keywords: hippocampus, propofol, thiopental, EPSC, IPSC
Introduction

GABA<sub>A</sub> receptors are GABA-operated chloride channels that produce inhibition in at least two modes: via relatively short lived ("phasic") fast direct inputs at synapses (e.g. inhibitory post-synaptic potentials; referred to here as "GABA<sub>phasic</sub>") and via induction of a sustained, background form of inhibition ("tonic" GABA<sub>A</sub> conductances; referred to here as "GABA<sub>tonic</sub>") (Mody 2001). While important roles for GABA<sub>phasic</sub> are well established, roles of GABA<sub>tonic</sub> are poorly understood, and the molecular identity and location of receptors underlying GABA<sub>tonic</sub> are unknown in most brain regions, with the notable exception of cerebellar granule cells (Brickley et al. 1996; Hamann et al. 2002). Recent evidence indicates that GABA<sub>tonic</sub> is present in many brain areas and has an important role in controlling the input-output properties of at least some neurons (Brickley et al. 1996; Hamann et al. 2002; Semyanov et al. 2003). There is also emerging evidence that GABA<sub>tonic</sub>, like GABA<sub>phasic</sub>, is activated/enhanced directly or indirectly by many clinically used drugs and some endogenous compounds, including some anesthetics: propofol (Bai et al. 2001); midazolam (Bai et al. 2001; Yeung et al. 2003); vigabatrin (Overstreet and Westbrook 2001); neuroactive steroids (Stell et al. 2003).

Anesthetics may act by altering fast synaptic input (i.e. enhancing inhibition or suppressing excitation) and/or suppressing the intrinsic excitability of neurons (i.e. depressing directly the ability of neurons to fire action potentials). This study is primarily focused on gaining a better understanding of the relative role (if any) of GABA<sub>tonic</sub> in producing depression of intrinsic neuronal excitability in neurons in intact acute brain slices, where the neuronal properties should be similar to in vivo. In the case of propofol, a widely used anesthetic, there are many sites of action that may affect intrinsic excitability, including effects on phasic (Trapani et al. 2000) and tonic (Bai et al. 2001) inhibition mediated by GABA<sub>A</sub>-Rs; sodium channels (Ratnakumari and Hemmings 1997; Rehberg and Duch 1999); potassium channels (Magnelli et al. 1992); cation channels (I<sub>h</sub> (Higuchi et al. 2003)); calcium channels (Guertin and Hounsgaard 1999; Inoue et al. 1999); glutamate channels (Orser et al. 1995); and glutamate release (Ratnakumari and Hemmings 1997). Assuming that propofol suppresses intrinsic excitability, two critical questions emerge: (1) Is GABA<sub>tonic</sub> enhancement
by anesthetics of sufficient magnitude to affect intrinsic excitability?; (2) Given the existence of other proposed sites of propofol action that would affect intrinsic excitability, are effects on GABA<sub>tonic</sub> relatively dominant, relatively unimportant, or one contributor among many? To our knowledge, there has been no direct investigation of the role of anesthetic enhancement of GABA<sub>tonic</sub> in affecting intrinsic neuronal excitability, and no attempts to determine the relative role of this mechanism.

Propofol acts on a wide variety of GABA<sub>A</sub> receptor subtypes to enhance GABA action and/or directly activate the receptors (Trapani et al. 2000; Williams and Akabas 2002). A recent analysis of mice with a point mutation in the GABA<sub>A</sub>-R beta3 subunit indicates that beta3-containing receptors play an important role in mediating propofol effects in vivo (Jurd et al., 2003), and receptors containing this mutation have very little potentiation by propofol (Siegwart et al. 2002). In a general sense, these results with beta3 mutants provide further strong evidence for an important role for GABA<sub>A</sub>-Rs in producing propofol anesthesia in vivo.

The hippocampus has been widely used for studies of anesthetics (MacIver 1997; Nicoll and Madison 1982) and appears to be an important locus for anesthetic action (Ma et al. 2002). In this study, we primarily investigated CA1 interneurons because these cells appear to play large roles in controlling CA1 network activity (Fricker and Miles 2001; Klausberger et al. 2003); hence, relatively small effects on these neurons are expected to have large effects on circuit rhythms, which are known to be disrupted during anesthesia (Maclver et al. 1996).

**Materials and Methods**

Brain slice preparation methods have previously been described in detail (Nishikawa and MacIver 2000). In short, standard transverse hippocampal slices (400-500 µm) from mature Sprague-Dawley rats (P28-P40, most P33-36) were prepared using a vibratome. All procedures conform to Stanford University and NIH guidelines.

**Electrophysiology**

Standard visualized slice procedures were used (Nishikawa and Maclver 2000). All interneuron recordings were from non-pyramidal CA1 neurons at or near the border of stratum radiatum and stratum lacunosum-moleculare; "giant" neurons (Gulyas et al. 1998) were
avoided. Interneurons were highly variable in cell size and shape. Interneurons had relatively non-accommodating action potentials, but varied in resting potential and threshold current required to produce spiking; there was no spontaneous spiking. Input resistances were often ~350-500 Mohm, but varied from ~200 Mohm to ~850 Mohm. However, there were no obvious differences between cells in response to propofol or thiopental: every interneuron displayed a decrease in both spiking and input resistance. Throughout current clamp experiments, sets of current steps were applied repetitively at fixed intervals (typically, 6 steps/set: 2 hyperpolarizing, one at zero pA, and 3 depolarizing); 1 set/minute). In long current clamp experiments, we found that neurons tolerated protocols using small numbers of depolarizing current steps repetitively applied (3/set) better than protocols involving sets with large numbers of depolarizing steps. We did not apply tonic currents to adjust resting potentials. Whole-cell recordings from pyramidal cells were from visualized neurons in stratum pyramidale. All experiments were conducted at room temperature (22-24°C) using a submersion chamber and > 95 % of tubing was Teflon to minimize drug binding. Using continuous perfusion of ACSF at 2-3 mL/minute complete bath replacement took ~2 minutes, as measured by dye exchange. Each slice was used for only a single experiment. The following external (ACSF) was used (in mM): 124 NaCl, 3.5 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 2 CaCl₂, 26 NaHCO₃, 10 glucose; it was bubbled with 95% O₂/5% CO₂ to reach pH 7.4. In some voltage clamp experiments, this solution was supplemented with 5 mM CsCl (to block Iₜ) and/or 50 µM CdCl₂ (to block calcium currents) to improve voltage clamping. When cadmium was used, the NaH₂PO₄ was eliminated. For high-K⁺ evoked spiking (Fig. 5), normal ACSF was supplemented with 6.5 mM KCl (replacing NaCl) to lead to a total of 10 mM KCl. We used standard whole-cell methods (pipette resistance 4-8 Mohm). For all current-clamp recordings (and voltage clamp recordings where indicated), a KGluconate-based internal solution was used (in mM): 100 KGluconate, 10 EGTA, 5 MgCl₂, 40 HEPES, 2 Na₂ATP, 0.3 NaGTP (pH 7.2 with KOH). For voltage-clamp experiments, this solution was supplemented with QX-314 (1-5 mM) to block sodium currents and, in experiments on evoked IPSCs (i.e. Fig. 4), the concentration of MgCl₂ was changed to 3 mM. KCl internal was:100 KCl, 10 EGTA, 5 MgCl₂, 40 HEPES, 2 Na₂ATP, 0.3 NaGTP, 1 QX-314 (pH 7.2 with KOH). For recording of cell-
attached spiking (Fig. 4), the pipette was filled with ACSF, a loose seal was formed on a visually identified interneuron and voltage clamp mode was used (Vcommand = 0 mV; (Bieda and Copenhagen 2000)). In most experiments on synaptically evoked spiking and glutamatergic inputs (Fig. 4), the CA1-CA3 connection and the CA1-subiculum connection were cut and a cut was often made at the border of stratum pyramidale and stratum radiatum to further isolate activity. For recording of extracellular spiking from CA1 pyramidal cells (Fig. 5), an ACSF filled pipette (6 MOhm) was placed in stratum pyramidale. All voltage values are corrected for electrode offset and junction potential. All recordings were established for >15 minutes before recording baseline data and recordings showing unstable response properties (> 15 % variability) were not used. To stimulate synaptic inputs, a bipolar tungsten electrode was placed in stratum radiatum, as previously described (Nishikawa and Maclver 2000).

Compounds

All compounds were from Sigma/RBI, except propofol, a kind gift of Zeneca Pharmaceuticals. Propofol was stored frozen as a 100 mM stock in DMSO under nitrogen gas in individual aliquots. Thiopental was made fresh daily as a 12.5 mM stock in distilled water and NaOH.

Data Collection and Analysis

Whole-cell and synaptically evoked spiking data (Figs. 1-4) were collected using PClamp 8.0 (Axon Instruments, Union City, CA) and analyzed using custom procedures written (by M.B.) in Igor Pro 3.15/4.0 (Wavemetrics, Oswego, OR). Extracellular spiking from stratum pyramidale was collected using DataWave (DataWave Technologies, Long Mount, CO); analysis was performed using this program’s spike discrimination and rate meter analyses; results were verified manually. The two-tailed Student’s t-test or repeated measures ANOVA with Tukey test were used to evaluate statistical significance (p<.05 or below) (GraphPad Prism software, Prism Inc., San Diego, CA, USA).

Results

Determination of the appropriate concentration of propofol for use in in vitro experiments, particularly brain slice experiments, has been quite difficult, with previous studies
using from 0.2 µM (Antkowiak 1999) to 500 µM (Wakasugi et al. 1999). We chose a nominal concentration of 30 µM for the majority of experiments based on several considerations, including: (1) the excellent correlation across a range of anesthetics (e.g. volatile and barbiturates) between \textit{in vitro} anesthetic concentrations that suppress CA1 field population spikes (fPS) and clinically relevant \textit{in vivo} anesthetic concentrations (MacIver 1997) coupled with our previous finding that 30 µM propofol suppresses the CA1 fPS to a similar degree as inhalational anesthetics at clinical concentrations (Turnquist et al. 2002); (2) the fact that the concentration of propofol in the slice at the end of our typical drug application period (40 minutes) is much lower than the applied bath concentration (5 to 30 µM) because steady-state propofol effects require over 300 minutes of application (see DISCUSSION for details) and (3) the desire to use an adequate concentration of propofol to assay for several possible previously described sites of action, which exhibit somewhat lower affinities for propofol (e.g. sodium channels (Ratnakumari and Hemmings 1997; Rehberg and Duch 1999)). Given these considerations, the actual effect-site concentration of propofol in these experiments is unknown, and it is possible that the effect-site concentration may be closer to levels used clinically for neuroprotection than levels used for anesthesia (i.e. 2 to 5 µM). Because further analysis of this question relies, in part, on the results presented in this report, we defer this topic to the DISCUSSION section.

\textbf{Propofol Strongly Depresses Interneuron Excitability}

Whole cell current clamp recordings from CA1 interneurons near the border of stratum lacunosum-moleculare were performed to assess the effect of propofol on cell excitability, as measured by spiking in response to current injection ("intrinsic excitability"). Propofol (30 µM), in a mostly reversible manner, strongly depressed both the ability of positive current steps to induce action potential production (data not shown; n=5 cells; spiking (spikes/s): control: 23.6 ± 1.3, propofol: 8.2 ± 1.4, wash: 17.5 ± 2.2; p<.001 control vs propofol) and the neuronal input resistance (equivalent to an increase in input conductance; n=5 cells, data not shown; conductance (nS): control: 2.4 ± 0.5, propofol: 3.63 ± 0.5, wash: 2.9 ± 0.6; p<.05 control vs propofol). Effects on spiking and input resistance developed slowly and roughly in parallel during a 35 minute propofol application. For comparisons between cells, the effect of propofol
on current injections leading to ~20 spikes/sec in control were analyzed. This approach provided a means of normalizing responses from cells with differing responses to current injections. Propofol produced a small hyperpolarization (n=5 cells; -3.0 ± 1.9 mV; not significant), which we did not attempt to compensate for with current injection. Hence, excitability suppression may be primarily due to shunting via conductance increases, although effects on other processes (e.g. sodium channels) could also play a role. This depression of intrinsic excitability is in strong contrast to our previous results with inhalational anesthetics (Nishikawa and MacIver 2000), which do not alter current evoked discharge in these neurons.

There is extensive evidence that propofol strongly enhances GABA_A receptor (GABA_A-R) function (Trapani et al. 2000). Application of the GABA_A channel antagonist picrotoxin (50 µM), in the continued presence of propofol, effectively reversed the effects of propofol on spiking and input conductance (Fig. 1A-C). These findings suggest that propofol’s depression of intrinsic excitability is largely due to activation of a GABA_A-R operated chloride channel, however, picrotoxin has been shown to depress other currents, including some cation channels (e.g. Das et al. 2003). It is also possible that this current is glycine-mediated or comes from a picrotoxin sensitive non-ligand gated chloride channel.

To determine more directly whether propofol induced a chloride conductance, as would be expected with activation of GABA_A receptors, we used an ionic replacement approach in voltage clamp experiments (Fig. 1D-F). Propofol produced a conductance increase with both low-chloride and high-chloride internal solutions, and we found that at ~60 mV, propofol produced an outward current with a low chloride (KGluc; E_Cl = ~65 mV) internal solution and a large inward current with a high chloride (KCl; E_Cl = ~5 mV) internal solution. These effects were largely reversed by picrotoxin. Overall (n=5 cells in each condition), propofol produced a small outward current in KGluc loaded cells (33 ± 11 pA) and a large inward current in KCl loaded cells (-198 ± 51 pA). With KCl internal solutions, we used short (~20 minute) applications of propofol because, in initial experiments, we observed significant cell-swelling with our typical (40-60 minute application) propofol protocol. It is important to note that these effects were not observed with Kgluconate internal solutions, which more closely reflect the internal chloride concentration of neurons in the intact brain. Therefore, the
quantified propofol-induced increases in inward current and conductance for KCl experiments are underestimates. To determine approximate minimum applied levels of propofol that activate this chloride conductance, we applied propofol at different concentrations for 20-30 minutes (KCl internal, Vh=-60 mV), to allow comparison to current measurements at 30 µM, and quantified the increase in current. We found significant currents at both 5 and 10 µM using this protocol (Fig. 1G; n=4 each concentration; 5 µM: -28 ± 7 pA; 10 µM: -61 ± 8 pA). For comparison purposes, the data for 30 µM propofol, which used an identical protocol, is replotted here (from Fig. 1F(left)). These results suggest that propofol at applied concentrations as low as 5 µM also activate this chloride conductance. Although glycine receptors are present in young adult rat (3-4 week old) hippocampal CA1 pyramidal cells and interneurons (e.g. Chattipakorn and McMahon 2002), propofol suppression of spiking and enhancement of conductance was found to be insensitive to the glycine receptor antagonist strychnine and sensitive to GABAA-R antagonist bicuculline, indicating that these propofol effects were due to GABAA-R, but not glycine receptors (Fig. 1H). In voltage clamp experiments testing strychnine and bicuculline, we used a 1:1 mixture of potassium gluconate and KCl internal solutions to avoid the problems encountered with the KCl solution in our previous voltage clamp experiments (see above text regarding Fig. 1D-F). Taken together, these results support the hypothesis that propofol activates a GABAA-R-associated chloride conductance at applied concentrations as low as 5-10 µM.

**Differential Pharmacology of GABA_A conductances**

A number of studies have examined block of GABA_A phasic and GABA_A tonic by SR95531 (Bai et al. 2001; Brickley et al. 2001; Hamann et al. 2002; Nusser and Mody 2002; Overstreet and Westbrook 2001; Semyanov et al. 2003; Stell and Mody 2002). All of these studies agree that 20 µM SR95531 is sufficient to completely block GABA_A phasic; however, some of these studies find that 20 µM SR95531 will strongly block GABA_A tonic, while others find that GABA_A tonic is insensitive to 20 µM SR95531. We examined the ability of this relatively high concentration of SR95531 (20 µM) to discriminate GABA_A phasic and GABA_A tonic with the logic that if a high concentration were sufficient to differentiate the populations, we could be assured of blocking GABA_A phasic while mostly preserving GABA_A tonic.
To test this differential pharmacology, we evoked monosynaptic IPSCs (recorded using 17.2 µM CNQX and 100 µM APV to block glutamate receptors) in neurons using stratum radiatum stimulation. To avoid problems associated with large propofol-induced chloride currents (see Fig. 1E and above text discussion), we used a low chloride (KGluc) internal solution. Assuming that SR95531 can be used to differentiate phasic and tonic GABA<sub>a</sub> conductances (Bai et al. 2001), we expected to observe a propofol-induced enhancement of the IPSC and the increase in holding currents we had already observed. This increase in holding current should be composed of both summing spontaneous IPSCs (which are augmented in propofol (Bai et al. 2001)) and a component due to GABA<sub>tonic</sub>. Thus, we expected that SR95531 would block a portion of the increase in holding current. Picrotoxin should block the remaining increase in holding current.

As expected, we found that propofol both enhanced the evoked IPSC and produced a background tonic current (Fig. 2). Additive application of SR95531 (20 µM) completely blocked IPSCs but only partially reduced the tonic current, while additive application of picrotoxin fully blocked the tonic current. Although the data appear to show that SR95531 only partially inhibits the tonic current for a short period of time (a "transient" block), a small sustained block of the tonic current could be masked by the slow rise of the tonic current, implying that in actuality SR95531 may be producing a sustained partial block of the tonic current. Most cells showed a smaller "transient" effect of SR95531 on the holding current than seen in Fig. 2B. All cells (n=6; one cell was lost before application of SR95531) showed a similar pattern of effects, with the exception of variable changes in IPSC amplitude. On average, the maximal (and "transient") effect of SR95531 reduced the total holding current by ~12% (I<sub>hold (total)</sub>: Propofol: 67 ± 9 pA; transient minimum in Pro + SR: 59 ± 10 pA), which corresponds to a decrease of ~20% in the net propofol-induced current. However, by the end of the SR95531 application period (15-20 minutes), the holding current had recovered to the same amplitude as in propofol alone (propofol: 67 ± 9 pA, propofol + SR: 68 ± 13 pA).

Overall, statistically significant differences were found at the p<.01 (or better) level for holding current (I<sub>hold</sub>) with the following comparisons: Con vs. +Pro, Con vs. +SR, +Pro vs. +Pic, and +SR vs. +Pic, and no significant (p>.05) differences were found for +Pro vs. +SR and Con vs.
In contrast to the effect on the eIPSC, propofol's enhancement of Ih was delayed and failed to saturate during the application period. This is consistent with a model in which there is slow propofol entry into the slice (Hollriegel et al. 1996; Turnquist et al. 2002) and a higher affinity of GABA\textsubscript{phasic} vs. GABA\textsubscript{tonic} for propofol. The SR95531-mediated partial and "transient" inhibition and the decrease in variance in Ih due to SR95531 may reflect blockade of background summating synaptic spontaneous IPSCs. Every individual cell showed a complete block of GABA\textsubscript{phasic} (both for spontaneous and evoked IPSCs) and at most a transient and partial block of GABA\textsubscript{tonic} by SR95531. These findings demonstrate that SR95531 and picrotoxin can be used to differentiate phasic and tonic GABA\textsubscript{A} conductances in hippocampal CA1 neurons.

**Role of GABA\textsubscript{tonic} in Propofol Neurodepression**

In whole-cell current clamp experiments (Fig. 3), SR95531 (20 µM, 15 minutes) failed to reverse the effects of propofol; in fact, the depression of excitability continued to increase during SR95531 application. Additive application of picrotoxin fully reversed propofol effects. Overall, statistically significant differences were found at the p<.001 level for both conductance and spiking for the following: Con vs +Pro, Con vs +SR, +Pro vs +Pic, and +SR vs +Pic, and no significant (p>.05) differences were found between +Pro vs +SR and Con vs +Pic. Hence, the effects of propofol on intrinsic excitability appeared to be either mostly or entirely due to enhancement of GABA\textsubscript{tonic}.

We next sought to determine whether propofol suppressed synaptically-evoked spiking in a similar manner. To avoid potential whole-cell dialysis effects and the forcing of intracellular chloride concentration to pipette concentration, we generally employed a cell-attached patch mode to monitor action potential production (Bieda and Copenhagen 2000). Electrical stimuli in stratum radiatum were adjusted so that 50-90% of trials resulted in spike production under control conditions (probability of spiking - P(s); percentage calculated using 20-30 consecutive trials in each condition).

In a representative experiment (Fig. 4A), propofol decreased P(s) in a picrotoxin-sensitive manner. Because similar results were found in all cells tested using either whole cell recording mode (n=5: Con: 66 ± 6%; Pro: 6 ± 3%; Pro + Pic: 96 ± 3%) or cell-attached
recording mode (n=5: Con: 69 ± 4%; Pro: 2 ± 1%; Pro + Pic: 94 ± 2%), these data sets are accumulated together in Fig. 4C (left). These data suggest that propofol depressed synaptically evoked action potential discharge via GABA\textsubscript{A} conductances and that whole-cell dialysis does not significantly affect propofol’s actions.

Given the powerful enhancement of IPSC charge transfer by propofol (see Fig. 4), propofol’s depressant effect in these experiments could occur primarily via enhancement of GABA\textsubscript{phasic}. If propofol primarily acts via GABA\textsubscript{phasic}, then SR95531 should largely reverse the depression by propofol, and further additive application of picrotoxin should have little effect. In a representative experiment (Fig. 4B), propofol’s actions were largely SR95531-insensitive and picrotoxin-sensitive. In all cells tested (n=5; Fig. 4C(right)), propofol strongly and consistently depressed P(s). Overall, statistically significant differences were found at the p<.05 (or better) level for the following: Con vs +Pro, Con vs +SR, +Pro vs +Pic, and +SR vs +Pic, and no significant (p>.05) differences were found with +Pro vs +SR and Con vs. +Pic, indicating that effects were overall SR95531-insensitive and picrotoxin-sensitive. In addition, in all cases (5/5), application of picrotoxin in the presence of SR95531 increased P(s), and in 80% (4/5) of cells, P(s) in picrotoxin was much greater than in SR95531 (means for these four cells: Con: 70 ± 5%; +Pro: 9 ± 5%; +SR: 18 ± 7%; +Pic: 90 ± 6%). These strong picrotoxin effects coupled with the weak and statistically non-significant SR95531 effects indicate that, under our conditions, suppression of synaptically-evoked spiking by propofol occurs largely via activation of GABA\textsubscript{tonic}. However, these results must be interpreted with caution; for in vivo conditions, where the ratio and timing of IPSPs and EPSPs could be different, GABA\textsubscript{phasic} may play a much larger, even dominant role.

Propofol has been described as suppressing glutamate exocytosis (Lingamaneni et al. 2001; Ratnakumari and Hemmings 1997) and potentially could affect AMPA/K receptors on interneurons. The protocols employed in Fig. 4A-C could fail to reveal these effects, because a large increase in P(s) by elimination of GABAergic inhibition could overwhelm a small decrease in P(s) produced by a weaker suppression of glutamatergic transmission. To test this possibility, we evoked pure glutamatergic EPSCs and EPSPs in interneurons using stratum radiatum stimulation and picrotoxin (100 \textmu M) to block GABA\textsubscript{A}-mediated responses.
We found that propofol (30 µM, 40 minutes) did not affect the amplitude of glutamatergic synaptic responses (EPSCs or EPSPs; Fig. 4D-F). Therefore, these results suggest that propofol does not affect glutamate release or AMPA/K receptors on these cells.

**Propofol Suppresses CA1 Pyramidal Cell Excitability**

A selective strong suppression of inhibitory interneurons should be strongly proconvulsant, which is not consistent with anesthetic actions. This effect would be prevented by a propofol suppression of excitatory neurons in the circuit. Hence, we tested the effects of propofol on CA1 pyramidal cells. Propofol induces a tonic GABA<sub>A</sub>-R mediated current in CA1 pyramidal cells (Whittington et al. 1996). Using a KCl-based solution in voltage clamp (n=3; protocol as in Fig. 1E), we found that propofol (30 µM) induced a conductance (Control: 4.2 ± 0.8 nS; Propofol: 7.5 ± 0.6 nS; p<.05) that was largely picrotoxin-sensitive (to 5.2 ± 0.9 nS). Propofol induced a significant inward current (at -60 mV, -167 ± 21 pA) consistent with a chloride conductance. These results indicate that propofol also suppressed CA1 pyramidal cell excitability via activation of GABA<sub>A</sub> conductances.

Hippocampal pyramidal cells often show strong spike adaptation due to activation of calcium-activated potassium channels (Madison and Nicoll 1986); the activity of these channels would probably be greatly affected by whole-cell dialysis in our long (~80 minute) experiments. To avoid this problem, we induced spiking by continuously applying an external solution containing 10 mM potassium and performed extracellular multiunit recordings in the CA1 stratum pyramidale. GABA<sub>A</sub> receptor activity was isolated by applying a combination of CNQX/APV/CGP55845A to block glutamate and GABA<sub>B</sub> receptors. Bicuculline (10 µM) fully reversed propofol (10 µM) suppression of pyramidal cell excitability (propofol: 19 ± 5% of control; bicuculline + propofol: 104 ± 8% of control; data not shown), implicating GABA<sub>A</sub> conductances. In all subsequent experiments, we added SR95531 to all solutions to block GABA<sub>phasic</sub> and used 10 µM propofol. Propofol produced a picrotoxin-sensitive depression of multiunit and single-unit activity that slowly developed during lengthy (60 minutes) applications, but did not appear to affect the size or shape of action potentials, suggesting that activation of GABA<sub>tonic</sub> by propofol can strongly reduce the intrinsic excitability of CA1 pyramidal neurons (Fig. 5).
A Role for GABA_{tonic} in Thiopental Neurodepression

To determine whether other anesthetics might be able to suppress intrinsic excitability via GABA_{tonic}, we tested thiopental, a widely used intravenous barbiturate anesthetic. Thiopental has been reported to induce a sustained GABA_{A-R} mediated current in pyramidal cells (Lukatch and Maclver 1996; Whittington et al. 1996). Thiopental at a concentration of 80 µM produces deep anesthesia in rats (Maclver et al. 1996) and depresses CA1 fPS to a similar extent as volatile anesthetics at clinical concentrations, as well as 30 µM propofol (Maclver 1997; Turnquist et al. 2002). As with propofol, we found that thiopental (80 µM) strongly but slowly depressed both neuronal excitability and input resistance in a largely SR95531-resistant and picrotoxin-sensitive fashion (n=5 cells; data not shown; spiking (Hz):

Con: 20.5 ± 1.2, thiopental: 6.4 ± 1.4, thiopental + SR: 4.7 ± 3.2, thiopental + SR + picrotoxin: 21.1 ± 1.8; conductance (nS): Con: 2.5 ± 0.3, thiopental: 3.7 ± 0.4, thiopental + SR: 4.1 ± 0.4, thiopental + SR + picrotoxin: 1.9 ± 0.3). Overall, statistically significant differences were found at the p<.001 level for both conductance and spiking with the following comparisons ('TP' denotes thiopental): Con vs +TP, Con vs +SR, +TP vs +Pic, and +SR vs +Pic, and no significant (p>.05) differences were found with +TP vs +SR. A small, but significant (p<.05) difference was observed between Con and +Pic. These results indicate that thiopental, like propofol, depressed neuronal excitability and enhanced input conductance by activating GABA_{tonic}.

Discussion

These data represent, to our knowledge, the first direct demonstration that anesthetics can suppress intrinsic neuronal excitability, as measured by action potential production, by activating GABA_{tonic}. We found that, under our conditions, suppression of intrinsic excitability by propofol was completely reversed by blockade of GABA_{tonic}; hence, this conductance appears to play a dominant role in producing this anesthetic-induced suppression of intrinsic excitability in these neurons. However, for in vivo conditions, effects on sites that alter synaptic input (in particular, direct enhancement of GABA_{phasic} as in Fig. 2) will play important roles, and assessing the relative importance, overall, of GABA_{tonic} in
producing anesthesia warrants further study. Generally, suppression of intrinsic excitability should have a variety of effects, including suppression of global firing activity, a reduced probability of long-term potentiation induction and depression of rhythmic neuronal activity. Disruption of rhythmicity by propofol or thiopental has been observed in vivo (MacIver et al. 1996; Tomoda et al. 1993) and in brain slices (Lukatch and MacIver 1996; Whittington et al. 1996); these effects may be due to effects on GABA\textsubscript{tonic}, but effects on GABA\textsubscript{phasic} would also certainly contribute. We anticipate that future directions relating to this work will focus on evaluating the role of GABA\textsubscript{tonic} in producing anesthesia in vivo, generalizing the results in this study to other anesthetics, other brain regions (especially spinal cord, thalamus and neocortex) as well as determining the properties of GABA\textsubscript{tonic}.

Previous studies have described several sites of action for propofol (see INTRODUCTION). Our propofol concentrations (5, 10 & 30 µM) and experimental protocols (direct current injection, synaptically-evoked spiking, high-K+ evoked spiking; use of both whole-cell and extracellular recording) were designed to allow detection of effects of these previously proposed sites for propofol depression of intrinsic excitability. None of these other sites appeared to play an important role in producing propofol effects on intrinsic excitability, but might contribute to actions on synaptic transmission. Alternatively, roles of other mechanisms in suppressing intrinsic excitability may occur with higher propofol concentrations and/or in other brain regions. Our results suggesting a powerful role for GABA\textsubscript{A} conductances are consistent with recent in vivo evidence showing that a point mutation in the beta-3 GABA\textsubscript{A} subunit can markedly attenuate propofol anesthesia in mice (Jurd et al. 2003). Our data strongly and clearly implicated GABA\textsubscript{tonic} in mediating propofol’s suppression of intrinsic excitability. In the case of synaptically evoked spiking, exact discrimination of relative roles of phasic and tonic GABA\textsubscript{A} conductances in propofol action is complicated by the expected synergistic action of propofol at these two sites, variability in the amount of synaptic inhibitory input into interneurons, and effects from the timing of action potential production during EPSP-IPSP sequences. Further research will be needed to understand better the roles of GABA\textsubscript{phasic}, GABA\textsubscript{tonic}, and other mechanisms in producing anesthesia in vivo. Interestingly, sedation, which occurs with low concentrations of propofol (much lower than surgical
concentrations of propofol), appears to be SR95531-sensitive, and may occur primarily via enhanced $\text{GABA}_\text{phasic}$ (Nelson et al. 2002). However, because high concentrations of SR95531 can also block $\text{GABA}_\text{tonic}$ (Nusser and Mody 2002; Yeung et al. 2003), linking sedation to actions on $\text{GABA}_\text{phasic}$ is not straightforward.

### Propofol Action at $\text{GABA}_\text{tonic}$

Several distinct mechanisms potentially underlie propofol enhancement of tonic $\text{GABA}_\text{A}$ conductances. Both propofol potentiation of GABA action at $\text{GABA}_\text{A}$ receptors and direct propofol activation of $\text{GABA}_\text{A}$ receptors have been extensively documented (Trapani et al. 2000; Williams and Akabas 2002) and can occur at quite low propofol concentrations ($\leq 1 \mu M$) in dissociated CA1 pyramidal cells (Hara et al. 1993, 1994). Propofol enhancement of GABA release (Hollrigel et al. 1996; Murugaiah and Hemmings 1998) could increase the tonic GABA concentration and thereby increase tonic $\text{GABA}_\text{A}$ conductance. Differentiation of these mechanisms will require a better understanding of $\text{GABA}_\text{tonic}$, but there is currently a limited understanding of the activation properties, function, and identity of the receptors producing $\text{GABA}_\text{tonic}$. A recent report (Semyanov et al. 2003) indicates that, in agreement with our results, $\text{GABA}_\text{tonic}$ exists in both CA1 pyramidal cells and CA1 interneurons. The low background concentration of GABA (0.8-2.9 $\mu M$) (Lerma et al. 1986), and the sustained nature of the current imply that the receptors producing $\text{GABA}_\text{tonic}$ are probably relatively high affinity and relatively non-desensitizing.

A point mutation in the $\text{GABA}_\text{A}$-R beta3 subunit (N265M) nearly abolishes propofol action at beta3-containing receptors (Siegwart et al. 2002). Mice with this beta3 (N265M) receptor show much reduced sensitivity to propofol (Jurd et al. 2003), indicating an important role for beta3 mechanisms. However, the continued (but depressed) sensitivity to propofol of these mice in the loss of righting reflex assay suggests that non-beta3 receptors probably also play a role. Mice with a beta2 mutation (N265S) still show propofol effects (Reynolds et al. 2003), and propofol still potentiates GABA action at receptors containing beta2 subunits with mutations of this residue (N265M; Siegwart et al. 2003), so these results are difficult to interpret for propofol. Hippocampal $\text{GABA}_\text{tonic}$ cannot be the same as cerebellar granule cell extrasynaptic $\text{GABA}_\text{A}$ conductances, because the cerebellar $\alpha_6$-containing receptors are not
found in hippocampus (Sperk et al. 1997; Wisden et al. 1992) and are blocked by SR95531 (Brickley et al. 2001). The receptor-channels producing \( \text{GABA}_{\text{tonic}} \) in CA1 may differ from those producing \( \text{GABA}_{\text{phasic}} \) in conductance (Bai et al. 2001) and kinetic and modulatory properties (Banks and Pearce 2000). In CA1 pyramidal cells, the tonic conductance is produced by receptors containing alpha5 subunits but not delta subunits; these receptors are most likely alpha5beta3gamma2 (Caraiscos et al. 2004). Hence, in our experiments on pyramidal cells, propofol is probably acting via these receptors. For interneurons, the composition of the \( \text{GABA}_{\alpha}-\text{Rs} \) is much less clear. Interneurons are highly variable, express a variety of \( \text{GABA}_{\alpha} \) subunits (at least alpha1, beta2, delta, gamma2; Sperk et al. 1997), and their \( \text{GABA}_{\alpha}-\text{R} \) subunit composition has not been studied as extensively as that in pyramidal cells. However, alpha5 and beta3 subunits are not prominent in interneurons (Sperk et al. 1997), so the interneuron tonic conductance probably differs in molecular composition from that of pyramidal cells. Some interneurons do contain delta subunits (Sperk et al. 1997), which confer high GABA sensitivity to extrasynaptic/perisynaptic receptors that produce tonic currents in dentate granule cells and cerebellar granule cells (Stell et al. 2003). However, because these subunits do not appear to be found in all interneurons (Sperk et al. 1997) but we observed strong propofol effects in all interneurons, it is probable that, as in pyramidal cells, gamma subunits may be important components of the \( \text{GABA}_{\alpha}-\text{Rs} \) underlying the propofol-induced tonic conductance in some interneurons.

We are not aware of an expressed \( \text{GABA}_{\alpha}-\text{R} \) that is SR95531-insensitive and picrotoxin-sensitive. \( \text{GABA}_{\text{tonic}} \) may simply represent a monoliganded state of receptors with the same molecular composition as phasic receptors for some interneurons (Bai et al. 2001) or the unbinding rate for SR95531 may be too slow to permit phasic receptors to become unblocked during the short time that GABA is present in a synaptic cleft (Jones et al. 1998). Currently, it is difficult to discriminate between these possibilities.

**\( \text{GABA}_{\text{tonic}} \) in other cells and with other anesthetics**

We found that the excitability of both GABAergic hippocampal interneurons and glutamatergic CA1 hippocampal pyramidal cells was strongly suppressed by propofol activation of \( \text{GABA}_{\text{tonic}} \), suggesting that other neuronal types may be affected similarly.
Several lines of evidence suggest that GABA_\text{tonic} may be a general locus of action for some, but not all, types of general anesthetics. We found that thiopental, a barbiturate general anesthetic, chemically unrelated to propofol, also strongly suppressed interneuron intrinsic excitability via GABA_\text{tonic}; however, anesthesia produced by thiopental \textit{in vivo} probably involves a strong synaptic component involving other mechanisms. Addition of various anesthetics, including barbiturates (methohexital (Zhang et al. 1998); pentobarbital (Uchida et al. 1996)), steroid anesthetics (alphaxalone (Ueno et al. 1997)), and other anesthetics (etomidate (Uchida et al. 1996)), produces currents or hyperpolarizations that are bicuculline-sensitive and relatively insensitive to SR95531. Hence, several other general anesthetics may target GABA_\text{tonic}; however, this has not been shown directly and the relative importance of GABA_\text{tonic} for depression of intrinsic excitability for these other anesthetics is unknown. The benzodiazepine midazolam only weakly potentiates GABA_\text{tonic} (Bai et al. 2001), but it will be important to determine whether this site plays a role in anesthetic-mediated cell death (Jevtovic-Todorovic et al. 2003). In contrast to these results, investigation of volatile anesthetics (e.g. halothane and isoflurane) has failed to reveal cellular effects consistent with activation of GABA_\text{tonic} (Nishikawa and MacIver 2000).

**Concentration of Propofol**

Measuring directly the free concentration of propofol in the brain during various states of anesthesia is not currently possible and is complicated by propofol’s high protein binding (Servin et al. 1988), lipophilicity (Tonner et al. 1992), and preferential partitioning into brain tissue (Shyr et al. 1995). Previous brain slice studies have used from 0.2 µM (Antkowiak 1999) to 500 µM (Wakasugi et al. 1999). We found propofol effects at 5 and 10 µM (Figs. 1G, 5). There is strong evidence that the propofol concentration in a brain slice is significantly less than applied perfusate concentrations. First, our experiments consistently show strong evidence for slow propofol entry - propofol effects developed slowly and often continued to increase after 40-60 minutes of application time (e.g. Figs. 2Btop, 5). Second, suppression of CA1 fPS amplitude by 5 µM propofol continues to increase even after >210 minutes of application (Turnquist et al. 2002), in contrast to the rapid onset and stabilization of effects in isolated neurons (e.g. <0.5 minutes) (Bai et al. 2001; Hara et al. 1993). Third, slices often
exhibit a high diffusional/binding barrier for entry of compounds (Dunlap et al. 1994), which should be particularly important for propofol, given its strong protein binding and high lipid solubility. Finally, a previous study (Hollrigel et al. 1996) also concluded that propofol enters brain slices slowly and that actual concentrations in the slice are almost certainly lower than perfusate concentrations, over the time course of our studies. Hence, the actual free effect site concentration of propofol acting within 30-40 min in our experiments is probably much lower than the applied concentration. An in vivo study in rats (Shyr et al. 1995) indicates that clinically relevant active site concentrations during anesthesia may be at least 10 µM, a concentration which activated GABA_{tonic} (Figs. 1G, 5) in our experiments. We did not test concentrations higher than 30 µM because this concentration already produced profound neurodepression. In part to address concentration issues, we also investigated thiopental, for which there is better pharmacodynamic and pharmacokinetic data. Thiopental was studied using 80 µM, a concentration of thiopental previously described as corresponding to deep anesthesia in rats (Maclver et al. 1996). This anesthetic also depressed neuronal excitability with a similar time course in our experiments.

In conclusion, enhanced GABA_{tonic} is likely to play an important role in the CNS depression that is associated with propofol and thiopental anesthesia. The depression of intrinsic neuronal excitability produced by these anesthetics would lead to decreased discharge frequencies of both pyramidal cells and inhibitory interneurons – disrupting the circuit functions of hippocampal neurons. This could contribute to the loss of recall, suppression of sensorimotor integration, and hypnosis that is produced by anesthetics.
Acknowledgments:

We acknowledge excellent technical support from Frances Monroe and Patricia Turnquist. We thank Dr. Martin Angst for aid in statistical analysis and helpful discussion regarding pharmacokinetic and pharmacodynamic reasoning, and Dr. James Trudell for advice on use of thiopental. Current address for Mark Bieda is: Santa Fe Institute, 1399 Hyde Park Rd., Santa Fe, NM 87501.

Grants

Research supported by NIH grant NIGMS 54767.
References

Antkowiak B. Different actions of general anesthetics on the firing patterns of neocortical neurons mediated by the GABA(A) receptor. *Anesthesiology* 91: 500-11, 1999.


Figure Legends

Figure 1. The intravenous anesthetic propofol strongly decreases neuronal excitability via induction of a GABA_A conductance in CA1 interneurons. (A) Top row shows amount of current injected; side markings indicate drug application. Propofol (30 µM; +Pro) fully suppressed spiking at +30 pA and partially suppressed spiking at +60 and +90 pA. Propofol effects were completely reversed by picrotoxin (+Pic). One second long current steps were applied. (B) Propofol decreased input resistance in a picrotoxin reversible fashion (-40 pA current injection). Same cell as (A). (C) Summary of data from five cells. left: Spiking; for comparison, responses to current steps leading to ~20 Hz in control are quantified. right: Conductance (G, nanosiemens). (D-F) Voltage clamp recordings from CA1 interneurons. (D) Low chloride (KGluc-onate-based) internal solution. Propofol induced a small outward current at ~60 mV and an increase in conductance. Steps from -60 mV to -90 mV in 10 mV increments. (E) High Chloride (KCl-based) internal solution. Propofol induced a large inward current at ~60 mV and a large increase in conductance. Steps from -50 mV to -90 mV in 10 mV increments. Series in (D) and (E) represent averages of three consecutive sets in each condition. Note scale difference between (D) and (E). (F) left: Summary of propofol-induced current at ~60 mV in KGluc-onate-loaded cells (n=5 cells) and KCl-loaded cells (n=5 cells). middle: Conductance (KGluc-loaded cells, n=5) right: Conductance (KCl-loaded cells, n=5). (G) Propofol-induced current at different concentrations. KCl internal. (H) Propofol acts via GABA_A conductances but not glycine conductances, because effects were blocked using the GABA_A-R antagonist, bicuculline (+Bic; 10 µM), but were not effected by the glycine antagonist strychnine (+Str; 2 µM): (1) Spiking (n=3 cells), (2) Holding current at ~70 mV (from voltage clamp experiments; n=4 cells), (3) Conductance (n=7 cells; n=3 in current clamp, n=4 in voltage clamp). Markings: “Con”: control; “+ Pro”: 30 µM propofol; “+Pic”: propofol (30 µM) + picrotoxin (50 µM); “+Str”: propofol (30 µM) + strychnine (2 µM); “+Bic”: propofol (30 µM) + strychnine (2 µM) + bicuculline (10 µM); “KGluc”: KGluc-onate-based internal solution; “KCl”: KCl-based internal solution. “****”: p<.001; “***”: p<.01; “**”: p<.05. Scalebars: (A-B) 30 mV, 200 ms. Dashed line (A) -50 mV. Resting membrane potentials in (A) were -59, -65, and -60 mV for Con, +Pro and +Pic, respectively.
Figure 2. Differential pharmacology of phasic and tonic GABA_{A} conductances. (A)
Monosynaptic electrically evoked IPSCs (17.2 µM CNQX and 100 µM APV present in all solutions; V_{h}=-49 mV; KGluc internal). Propofol (30 µM) enhanced monosynaptic IPSCs and produced a tonic background current. Addition of SR95531 (15 minutes, 20 µM) eliminated the IPSC but not the tonic current. Additive application of picrotoxin (50 µM) eliminated the tonic background current. (B) Timecourse of responses shown in (A). Note slow continuous development of tonic current (bottom) over 40 minutes. Note partial depression by SR95531; this depression appears “transient” but may reflect a sustained partial block of tonic current or suppression of sIPSC contributions to tonic current (see RESULTS). (C) Summary of all experiments (6 cells total: n=6 for Con and Pro; n=5 for SR and Pic). 1: Amplitude of tonic current. Tonic current in SR95531 measured at end of 15-20 minute application. For quantification of partial block observed during initial phase of SR95531 application, see RESULTS. 2: Amplitude of IPSC. 3: Charge transfer by IPSC (excluding tonic current). 4: Half-width of IPSC. Figure markings: “+Pro”: 30 µM propofol; “+SR”: 30 µM propofol + 20 µM SR95531; “+Pic”: propofol (30 µM) + SR95531 (20 µM) + picrotoxin (50-100 µM). “***”: p<5E-6; “**”: p<.001; “*”: p<.01; NS: not significant.

Figure 3. Propofol suppresses CA1 interneuron excitability via a tonic GABA_{A} conductance. (A) Suppression of spiking by propofol is reversed by picrotoxin (50 µM, 15 minutes) but not by SR95531 (20 µM, 15 minutes). (B) Propofol-induced conductance increase is reversed by picrotoxin but not by SR95531. -60 pA current injection. Same cell as (A). (C) Summary (n=5 cells). left: Spiking. For comparison, current injections leading to ~10 spikes/500 ms are summarized. right: Conductance. Scalebars: (A) 30 mV, 400 ms; (B) 10 mV, 500 ms. Other figure markings as in Fig. 1. Dashed lines: (A) -50 mV; (B) -90 mV. Resting membrane potentials in (A) were -60, -61, -60 and -61 mV for Con, +Pro, +SR and +Pic, respectively.
Figure 4. Propofol (30 µM) suppresses synaptically evoked spiking via action on a tonic GABA_{A} conductance but not action on AMPA/K receptors or glutamate release. (A) Cell-attached recording from CA1 interneuron. A bipolar stimulus electrode was placed in stratum radiatum about 200 µM from the interneuron. First upward event is electrical artifact. Subsequent bipolar event is single spike. Drug condition and percentage of trials when spike was produced indicated above traces. Effects of propofol developed over 40 minute period. Additive application of picrotoxin reverses effects of propofol. (B) Block of a tonic GABA_{A} conductance, but not phasic GABA_{A} conductances, reverses propofol effects. (C) Summary. left: Summary of experiments using only picrotoxin (n=10). This data set includes n=5 in cell-attached mode and n=5 in whole-cell mode; results were very similar and are combined here. right: Summary of experiments (n=5) employing both SR95531 and picrotoxin. Phasic GABA_{A} conductances had a variable contribution to suppression. (D-F) Propofol has little effect on glutamate release or AMPA/K receptors. All experiments performed in continuous presence of picrotoxin (100 µM). (D) Glutamatergic EPSCs recorded at -74 mV. (E) Timecourse of responses from cell shown in (D). (F) Summary (n=5 experiments; n=3 in voltage clamp and n=2 in current clamp). Figure markings as in Fig. 1 except: in (A) and (C), “+Pic”: 30 µM propofol + picrotoxin (50-100 µM); “∗”, p<.01; “∗∗”: p<.001; “∗∗∗”, p<10E-8. Scalebars: (A) 40 ms, 10 pA; (B) 40 ms, 8 pA; (D) 40 ms, 20 pA.

Figure 5. Propofol (10 µM) suppresses CA1 pyramidal cell excitability. Extracellular multiunit recordings from CA1 pyramidal cell layer using 10 mM K+ external solution. All recordings done in continuous presence of glutamate receptor blockers (17.2 µM CNQX and 100 µM APV), GABA_{B} receptors (5 µM CGP55845A), and phasic GABA_{A} conductances (10 µM SR95531). (A) Representative recording. Each set is 6 consecutive sweeps (500 ms) from a single experiment. Unit 1 is large single unit and Unit 2 is small single unit. (B) Timecourse of experiment in (A). Shaded portion shows overall multiunit activity (left axis scale). Single lines (right axis scale) show activity of unit 1 (thin line) and unit 2 (thick line). Note slow onset of propofol effect. (C) Action potential waveform was not altered by propofol. Five records from
control and five from propofol overlaid in each panel. (D) Summary of multiunit data from 8 slices. “*”: p<.001. All recordings used a concentration of 10 µM propofol.
Figure 1
Figure 2
Figure 5

A. Waveforms showing the effects of Con, +Pro (10 μM), and +Pic on Units 1 and 2.

B. Graph illustrating the multi-rate response over time with Con (+ CNQX + APV + SR + CGP), +Pro, and +Pic.

C. Voltage traces for Unit 1 and Unit 2.

D. Histogram showing the rate in Hz for Con, +Pro, and +Pic.