Propofol-block of SK Channels in Reticular Thalamic Neurons Enhances GABAergic Inhibition in Relay Neurons

Running Title: PROPOFOL BLOCK OF SK CHANNELS IN RTN

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ABSTRACT

The GABAergic reticular thalamic nucleus (RTN) is a major source of inhibition for thalamocortical neurons in the ventrobasal complex (VB). Thalamic circuits are thought to be an important anatomic target for general anesthetics. We investigated presynaptic actions of the intravenous anesthetic propofol in RTN neurons, using RTN-retained and RTN-removed brain slices. In RTN-retained slices, focal and bath-application of propofol increased intrinsic excitability, temporal summation, and spike firing rate in RTN neurons. Propofol-induced activation was associated with suppression of medium-afterhyperpolarization potentials. This activation was mimicked and completely occluded by the small conductance calcium-activated potassium (SK) channel blocker apamin, indicating that propofol could enhance RTN excitability by blocking SK channels. Propofol increased GABAergic transmission at RTN-VB synapses, consistent with excitation of presynaptic RTN neurons. Stimulation of RTN resulted in synaptic inhibition in postsynaptic neurons in VB, and this inhibition was potentiated by propofol in a concentration-dependent manner. Removal of RTN resulted in a dramatic reduction of both spontaneous postsynaptic inhibitory current frequency and propofol-mediated inhibition of VB neurons. Thus, the existence and activation of RTN input were essential for propofol to elicit thalamocortical suppression; such suppression resulted from shunting through the postsynaptic GABA$_A$ receptor-mediated chloride conductance. The results indicate that propofol enhancement of RTN-mediated inhibitory input via blockade of SK channels may play a critical role in “gating” spike firing in thalamocortical relay neurons.
INTRODUCTION

The thalamic reticular nucleus (RTN) is a thin sheet of GABAergic neurons that surrounds the anterior, lateral surfaces of the thalamus, and receives excitatory input from collaterals of both corticothalamic and thalamocortical axons (Jones 1985; Ohara and Lieberman 1985). RTN provides a major source of inhibitory input to thalamocortical neurons in the ventrobasal complex (VB) in rodents (Liu et al. 1995). Since VB lacks GABAergic interneurons, GABAergic inhibitory control of VB neuron responsiveness to excitatory inputs is primarily mediated through feed-forward inhibition (cortex-RTN-VB), and feedback inhibition (RTN-VB, Sherman and Guillery 2001; Steriade et al. 1997). Through GABAergic inhibition, RTN has been implicated in several important functions, including influencing the efficacy of sensory input (Lee et al. 1994; Le Masson et al. 2002), altering thalamic relay responses to corticothalamic excitatory input (Ergenzinger et al. 1998; Hartings and Simons 2000; Temereanca and Simons 2004), gating selective attention (Guillery et al. 1998; Hartings et al. 2000; 2003; Weese et al. 1999), synchronizing thalamic oscillations (Steriade et al. 1997), and generating sleep spindles and slow-wave sleep (Steriade 2001). Recent evidence has indicated that through RTN-mediated intra-thalamic connections, intra-thalamic nuclei (e.g., VB, posterior complex, ventrolateral nucleus and intralaminar nuclei) can interact with each other, and this circuitry enables RTN to play a pivotal role in switching between waking and sleep states and regulating thalamic sensory and motor information processing (Crabtree 1999, Crabtree et al. 1998; Crabtree and Isaac 2002).

Propofol is an intravenous anesthetic with a chemical structure distinct from any other anesthetic, and is also a potent modulator of GABA_A-receptors (Trapani et al. 2000). Propofol has been shown to potentiate GABAergic responses in postsynaptic neurons in several brain regions.
regions (Bai et al. 1999; Bieda and MacIver 2004; Chen et al. 1999; Collins 1988; Inoue et al. 1999; Jurd et al. 2003; Kitamura et al. 2003; Manuel and Davis 1998; Peduto et al. 1991; Reynolds et al. 2003; Wakasugi et al. 1999). Although effects of GABAergic anesthetics including propofol are generally thought to be mediated by GABAergic mechanisms, such a concept is mainly based on the assessment of anesthetic effects on GABAergic currents in postsynaptic neurons or non-neuronal systems. The manner in which propofol directly influences presynaptic GABAergic neurons is largely unknown. Therefore, the present study investigated the effects of propofol on presynaptic GABAergic RTN neurons, using mouse brain slices. To assess the role of RTN in mediating anesthetic actions in the thalamus, we studied the ability of propofol to influence VB neuron spike firing in RTN-retained and RTN-removed slices. Preliminary results have been published in abstract form (Ying and Goldstein 2002).
METHODS

Brain slice preparation

Experiments were performed in accordance with institutional and federal guidelines. Mice (C57BL/6, P25-55) were anesthetized by halothane and decapitated. The head was immediately submerged in ice-cold carbogenated (95% O₂/5% CO₂) slicing solution, and the brain was rapidly dissected out. To prepare horizontal slices, the brain was sagittally cut into two halves along the midline; slices (240 or 300 µm) were horizontally cut on a microslicer (Leica VT 1000S, Wetzlar, Germany) using a sapphire blade (Leica) to yield smooth-surface slices. Thalamocortical (TC) slices were prepared as described (Agmon and Connors 1991) with a slight modification. Briefly, after the brain was dissected out, the rostral portion of the brain was cut at 45° or 55°; the rostral end of the brain block was glued to a homemade platform, and slices (300 µm) were cut. Slices were gently rinsed once in cold artificial cerebrospinal fluid (ACSF), and incubated in carbogenated ACSF at 34 °C for 1 hr for recovery, and then at 24 °C for at least another 1 hr before use. Horizontal slices were used in most experiments, and TC slices were used for recording of spontaneous activity in VB and for corticothalamic pathway stimulation. All slices had intact RTN connections, unless otherwise specified (see Results).

Electrophysiology

Current-clamp recordings were performed at 35 °C. Slices were perfused with carbogenated ACSF; neurons were visualized and identified using a Zeiss Axioskop (Jena, Germany) equipped with a 2.5× objective and 40× water immersion objective with a 2.4 mm working distance and IR-DIC optics. Recordings were made from neurons located in either the RTN or VB; all RTN neurons were located in either somatosensory sector or the anterior RTN (Cox et al. 1996; Ohara and Lieberman 1985; Guillery et al. 1998). The resistance of the pipette
was 3.5 – 6.0 MΩ when filled with internal solution. Tight seal (> 2 GΩ) was achieved by application of a small negative pressure, using a 1 ml-syringe. Access resistance (Rₐ) was compensated by up to 70%, and ranged from 10-14 MΩ after compensation; data were discarded if Rₐ > 15 MΩ. Steady-state input resistance was measured at a holding membrane potential level close to resting membrane potential (RMP) from the voltage response elicited by a small current pulse (-60 pA, 500 ms). Only neurons that showed a stable RMP negative to -60 mV, action potential overshoot of > 10 mV and input resistance > 150 MΩ were selected for study. Although cells so selected generally showed stable data records for up to 240 min, pharmacological tests were completed within 90 min to minimize the variation of responses. Liquid junction potentials (11.1-12.2 mV) were calculated, based on intracellular and bath solutions, using the software Junction Potential Calculator (Clampex 9, Axon Instruments, Union City, CA), and corrected online or offline. Voltage-clamp recordings of inhibitory postsynaptic currents (IPSCs) were made at 25 °C, using a Cs⁺-based internal solution (Goldstein et al. 2002). Neurons were voltage-clamped at a holding potential of -60 mV, except for those experiments in which various holding potentials were used to test the effect of electromotive driving force on miniature IPSC amplitude. Membrane current was low-passed filtered at 2 Hz, and membrane voltage at 5 kHz, and then digitized at 10 kHz using an Axopatch 200A amplifier connected to a DigiData 1200 interface (Axon). In some experiments a Multiclamp 700B amplifier connected to a DigiData 1322A interface (Axon) was used. Data were collected at least 10 min after whole-cell access to allow the pipette solution to equilibrate with the neuron.

**Extracellular and intracellular electrical stimulation**

To evoke synaptic responses in RTN neurons, a concentric bipolar tungsten electrode (FHC Inc., Bowdoinham, ME) was placed in either layer VI of the barrel cortex or the white
matter in TC slices (Pedroarena and Llinás 2001). To evoke inhibitory postsynaptic potentials or currents (IPSPs or IPSCs) in VB neurons, the stimulation electrode was placed in RTN (at somatosensory sector) or the internal capsule (Warren et al., 1997). Single pulses were delivered using a Master-8 pulse generator (A.M.P.I., Jerusalem, Israel) controlled by a PC, and stimulus intensity was controlled by a constant current stimulus isolator (World Precision Instruments, Sarasota, FL). Responses were considered monosynaptic if the latency jitter was less than 0.4 ms, and their rise times were consistent from trial to trial (3 trials). To confirm that responses were GABA<sub>A</sub>-receptor-mediated, a GABA<sub>A</sub> receptor antagonist (bicuculline 10 µM or gabazine 10 µM) was used to block responses in the presence of a GABA<sub>B</sub>-receptor antagonist (2-OH saclofen 100 µM or CGP55845 1 µM). To identify excitatory synaptic potentials (EPSPs), CNQX (20 µM) and D-AP5 (40 µM) were used to block evoked EPSPs, and the Na<sup>+</sup> channel blocker tetrodotoxin (500 nM) was used to block evoked action potentials. Intrinsic spike firing was initiated with intracellular direct current (DC) injection or rectangular current pulses (500 – 1000 ms). To study temporal summation of intrinsic subthreshold voltage responses at the soma, five depolarizing exponential (EPSP-shaped) current pulses were generated with the function: 

\[ f(x) = A \times (1 - \exp(-t/\tau_{\text{rise}})^n \times \exp(-t/\tau_{\text{decay}})) \]

where A is the amplitude of the current (200-300 pA), \( n (=5) \) is an integer, and \( \tau_{\text{rise}} \) and \( \tau_{\text{decay}} \) are rising (2 ms) and falling (20 ms) time constants, respectively (Magee 1998).

**Bath and focal application of drugs**

Drugs were generally applied by bath superfusion (unless otherwise noted) for at least 20 min at a flow rate of 3 ml/min prior to data collection. The perfusion system consisted of polytetrafluoroethylene (Teflon<sup>®</sup>) tubing and connectors. Propofol was freshly prepared in
DMSO and diluted with ACSF to 0.3 – 6 µM, which were within clinically relevant concentration ranges (Franks and Lieb 1998). The final concentration of DMSO was 0.1%, which had no effects on the cells examined. In some cases, a drug solution was focally ejected onto the soma through a puffer pipette connected to Picospritzer II (Parker Instruments, Fairfield, NJ) using a pico-ejection technique. The pipette tip was placed 30-40 µm away from and ~20 µm below the soma, and specific responses to a drug were verified by a corresponding antagonist applied by bath whenever possible. The pipette tip was checked after use to see whether it was clogged.

Solutions (in mM)

Slicing solution contained 2.5 KCl, 24 NaHCO₃, 1.25 NaH₂PO₄, 234 sucrose, 11 glucose, 10 MgSO₄, and 0.5 CaCl₂. ACSF (bath solution) for current-clamp recordings contained 124 NaCl, 26 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 1.2 MgCl₂, and 2 CaCl₂ and 11 glucose. Intracellular solution contained 130 K-glucurate, 5 NaCl, 2 MgCl₂, 10 HEPES, 0.5 EGTA, 2 ATP-K, 0.3 GTP-Na, pH adjusted to 7.25 with KOH. K-glucurate was used because the impermeant ion glucurate does not contribute to anesthetic-induced changes in RMP or I-V relationship (Nishikawa and MacIver 2000). Bath solutions were freshly prepared on the same experimental day. The bath solution for voltage-clamp contained 117 NaCl, 25 NaHCO₃, 3.6 KCl, 1.2 NaH₂PO₄, 1.2 MgCl₂, and 2.5 CaCl₂ and 11 glucose; osmolarity was adjusted to 300 mOsm with sucrose.

Chemicals

Propofol was from Aldrich (Milwaukee, WI) or TCI (Portland, OR). (+) Bicuculline, gabazine, 2-OH saclofen, (2S)-3-[(1S)-1-(3,4-dichlorophenyl) ethyl] amino-2-hydroxypropyl] (phenylmethyl) phosphinic acid (CGP55845), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX),
and D-2-amino-5-phosphopentanoic acid (D-AP5) were from Tocris Cookson (Ellisville, MO). Tetrodotoxin (TTX) was from Alomone Labs (Jerusalem, Israel).

**Intracellular biocytin filling**

Neurons from 30 mice were intracellularly filled with biocytin (0.5% in the pipette solution) to verify recording sites. After recording, slices were fixed for 24-72 hrs in phosphate buffer (PB) solution containing 4% paraformaldehyde, transferred to 20% sucrose solution in 0.1 M PB and resectioned to 60-100 μm. After endogenous peroxidases were blocked with phosphate-buffered 3% H₂O₂, the slices were incubated with biotinylated horseradish peroxidase conjugated to avidin (ABC-Elite, Vector Labs, Burlingame, CA), washed and incubated with DAB for 15 min. Filled neurons were visualized using IR-DIC optics and drawn as previously described (Kawaguchi 1993).

**Data and statistical analysis**

Data were analyzed using both Clampfit 9 and MiniAnalysis 6.0.4. To analyze temporal summation containing five responses, the peak of the first and fifth responses was measured from baseline, and expressed as ΔV₁ and ΔV₅, respectively; responses were calculated as % increase = [(ΔV₅ / ΔV₁) – 1] × 100. Temporal summation was defined as % increase in depolarization occurring at the soma during a train (Magee 1999). Action potential parameters including amplitude, threshold, duration, and rise/decay time were also obtained. For analysis of fast afterhyperpolarization (fAHP) followed by a single spike, 100 - 500 successive spikes were averaged, and fAHP peak amplitude was measured from the baseline to the maximum negative point (peak). For analysis of medium-duration AHP (mAHP), mAHP was integrated (AHP area), between the point at which the spike falling phase reached the baseline and the point that the negative voltage deflection returned to the baseline (Sourdet et al. 2003). For % spike firing
suppression in experiments with RTN stimulation, the average number of spikes was obtained from at least 3 trials; % inhibition was calculated as (F2 /F1 - 1) x 100, where F1 and F2 were the average number of spikes recorded before and during treatment (drug application or electrical stimulation), respectively. To study GABAergic synaptic transmission at RTN-VB synapses, stimulation intensity was set to evoke IPSCs in ~50% of the stimulations, as calculated from 30 stimuli (3 trials, each containing 10 sweeps), and evoked responses with deflections from baseline < 4× RMS noise were considered failures (Liu et al. 2001). The concentration-effect curves were fitted with the Hill equation: 
\[ y = \frac{V_{\text{max}} \times x^{nH}}{x^{nH} + k^{nH}} \]
where \( y \) is percent inhibition at the drug concentration \( x \), \( V_{\text{max}} \) is the maximal percent inhibition, \( k \) is the EC50 value (concentration needed for a half-maximum response), and \( nH \) is the Hill slope constant.

Statistical analyses were performed with Sigmastat V3.0 (SPSS, Chicago, IL) using t-test or one-way ANOVA with post hoc test where appropriate. Large IPSCs (presumed action potential-dependent events) were defined as IPSCs with amplitude >240 pA, since no IPSCs greater than this value were observed in the presence of the sodium channel blocker tetrodotoxin (500 nM) under our experimental conditions. Cumulative amplitude distributions for IPSCs were generated, and differences in amplitude distributions were tested by Kolmogorov-Smirnov test (MiniAnalysis V6.0.4) with a significant difference at \( P < 0.05 \). Data are expressed as mean ± SE.
RESULTS

Neurons in RTN were readily located and identified in live brain slices, using IR-DIC optics. In a number of instances the position of the recorded neuron was confirmed using biocytin labeling (data not shown). The distinct membrane properties of GABAergic RTN and glutamatergic VB neurons allowed us to identify the two types of neurons, as illustrated in Fig. 1A. In response to a hyperpolarizing current pulse, RTN neurons showed a very shallow depolarizing sag regardless of membrane potential level; they generally showed multiple, rhythmic rebound spike bursts at relatively hyperpolarized membrane potentials, with no slow after-burst depolarization potential (ADP). All these features were identical to previous observations (Debarbieux et al. 1998; reviewed by Destexhe and Sejnowski 2001), and the rebound burst pattern was consistent with that observed for type I neurons in the cat RTN in vivo (Contreras et al. 1992). In contrast, VB neurons displayed a large, slow depolarizing sag and slow ADP in response to the same protocol (Fig. 1A bottom, protocol not shown).

Propofol increases intrinsic excitability and spike firing in RTN neurons

Propofol has been shown to produce a sedative effect, accompanied by an increase in GABAergic neuronal activity in the ventrolateral preoptic nucleus, a component of a sleep-promoting pathway in vivo (Nelson et al. 2002). Here we investigated effects of propofol on intrinsic excitability in GABAergic RTN neurons. Tonic spike firing was initiated with a depolarizing direct current (Fig. 1Bi). Bath application of propofol increased the spike firing rate; an example trace is shown in Fig. 1Bii. Group data (n = 18) demonstrated that propofol increased RTN spike firing by 28 ± 4 and 72.8 ± 8% at concentrations of 0.6 and 3 µM, respectively (Fig. 1Biii). To test for a direct effect in RTN, propofol (3 µM in pipette) was
focally applied to the soma through a 1 MΩ-glass pipette. Focal application produced a similar effect (n = 5, not shown).

To study how propofol affected both spike firing patterns and input resistance that is associated with intrinsic excitability, intrinsic spike firing was initiated with depolarizing and hyperpolarizing current pulses. RTN neurons showed both tonic spike firing and rebound spike bursts in response to current pulse injections (Fig. 1Ci). The intrinsic voltage response was consistent with that observed for type I neurons in the cat RTN in vivo (Contreras et al. 1992). Input resistance was 320 ± 18 MΩ (n = 20) in control; focal application of propofol (0.6 and 3 μM) increased input resistance to 390 ± 12 MΩ and 429 ± 17 MΩ, respectively, accompanied with an increase in single spike firing and rebound spike bursts (Fig. 1Cii). The activating effects produced by bath and focal application of propofol were similar, and data were therefore pooled. Normalized group data for input resistance (n = 20) are shown in Fig. 1Civ.

**Propofol suppresses apamin-sensitive mAHP**

The above data raised the question of how propofol increased intrinsic excitability. When RTN neurons fired single spikes, the spikes were followed by fast afterhyperpolarization potentials (fAHPs; lasting 1-2 ms) and medium AHPs (mAHPs; lasting ~300 ms). Analyzing spikes in another 20 RTN neurons activated by focal propofol, we found that mAHP was suppressed (Fig. 2A). Comparison of differences in AHPs in the absence (control) and presence of propofol is shown in Fig. 2B. Other spike parameters, including the threshold for spike firing and fAHP amplitude, were not significantly changed.

As reported for hippocampal neurons, mAHP is mediated by small-conductance Ca²⁺-activated potassium channels (SK channels) that regulate tonic firing rate, and mAHP is highly
sensitive to the SK channel blocker apamin (Stocker et al. 1999). To investigate if the apamin-sensitive mAHP was involved in regulating responses to propofol, apamin (200 nM in pipette) was focally applied to the soma in another subgroup of RTN neurons. Apamin reduced mAHP, and increased both input resistance and tonic spike firing (Fig. 2C-G). As illustrated in Fig. 2D, apamin decreased mAHP in a manner similar to propofol. The addition of propofol (3 µM) during apamin application or co-application of the two compounds produced a similar voltage response (Fig. 2C-D). Group data are shown in Fig. 2E-G. Both propofol and apamin markedly decreased mAHP, increased spike firing and input resistance in a similar manner; there was no significant difference in effects produced by apamin alone and apamin plus propofol groups. This clearly demonstrated that the effect of propofol was mimicked and completely occluded by apamin. Thus, the increase in RTN neuron intrinsic excitability, and tonic spike firing during propofol application was primarily due to blockade of the apamin-sensitive SK channel.

**Propofol increases temporal summation in RTN neurons independently of excitatory synaptic input**

The above data indicated that propofol markedly increased input resistance in RTN neurons. Alterations in input resistance are known to critically influence temporal summation of subthreshold voltage responses and, in turn, action potential generation (Magee 1998). Whether propofol was capable of affecting subthreshold responses via intrinsic excitability was directly investigated; intrinsic voltage responses (in the absence of major synaptic inputs) were initiated with intracellular injection of EPSC-shaped current pulses (train of 5 pulses, 33Hz), as done for hippocampal neurons (Magee 1998). Under control conditions (no drug), we found that the intrinsic subthreshold voltage response was incrementally increased during a train (Fig. 3Ai),
with an increase in summation of 32.2 ± 4.1% (n = 15). Focal application of propofol (3 μM in pipette) increased the summation (Fig. 3Aii). A clear comparison of differences in summation prior to and during propofol application is shown in Fig. 3Aiii. Group data (Fig. 3Avi) demonstrated that propofol significantly increased summation of the intrinsic response (56.8 ± 5%, P < 0.001, t-test). Detailed analyses of integrative properties are summarized in Table 1.

In another subgroup of RTN neurons (n = 5), intrinsic voltage responses were initiated with the same intracellular EPSC-shaped pulses at more depolarized membrane potential (-60 to -58 mV). As shown in Fig. 3Bi, summation was associated with the occurrence of a single spike, and bath application of propofol increased the number of spikes to 6 (Fig. 3Bii). To examine whether the SK channel-mediated conductance might be involved, the same response (control) was initiated in another RTN neuron (Fig. 3Ci). Focal application of apamin (200 nM in pipette) resulted in a response identical to propofol (Fig. 3Cii); subsequent bath application of propofol had little effect on summation (Fig. 3Ciii). These data indicated that propofol could enhance intrinsic subthreshold responses in RTN neurons via blockade of the apamin-sensitive SK channel, and that this enhancement was independent of excitatory synaptic input.

As shown in Figs. 1, 3 and 4, propofol depolarized RTN neurons. Group data demonstrate that propofol depolarized the membrane potential by 2.6 ± 0.5 mV, and this change, although modest, was significant (Wilcoxon Signed rank test, P < 0.001, n = 68). The SK channel blocker apamin produced a nearly identical depolarizing effect on the membrane potential (P < 0.001, n = 24, data from Figs. 2, 3 and 4). Thus, the depolarization during propofol application appeared due to blockade of SK channels, consistent with other observations (Cingolani et al. 2002; Hallworth et al. 2003).
Propofol can enhance synaptic temporal summation through the suppression of SK channels

RTN receives massive excitatory synaptic input from the cortex (Steriade et al. 1997). Increased intrinsic excitability in thalamic neurons can enhance synaptic responsiveness to corticothalamic excitatory input (Pedroarena and Llinás 2001). We hypothesized, therefore, that propofol-mediated enhancement of intrinsic excitability might also alter synaptic temporal summation. Effects of propofol on EPSP summation in RTN neurons were investigated by extracellular stimulation of corticothalamic fibers (5 pulses, 33 Hz) in thalamocortical slices. We found that corticothalamic stimulation in the absence of drug consistently gave rise to EPSP summation (602%) at membrane potentials of -66 mV (Fig. 4Ai). Bath application of propofol (3 µM) depolarized the membrane potential by 3 mV, and increased synaptic temporal summation to 870% (Fig. 4Aii). Differences in synaptic summation in the absence (control) and presence of propofol are illustrated in Fig. 4Aiii. Group data (Fig. 4Aiv) clearly indicated that propofol significantly increased synaptic temporal summation in RTN neurons (P < 0.001, t-test, n = 15). The effects of propofol on integrative properties of synaptic responses are summarized in Table 1.

We next examined whether propofol could alter synaptic responses during SK channel blockade for at least three reasons. First, the SK channel-mediated conductance can contribute to synaptic efficacy by altering neuronal excitability in postsynaptic neurons (Borde et al. 1999). Second, excitatory input from the cortex persists, albeit reduced, during propofol application at concentrations of 0.3 to 3 µM (our unpublished observations; see also Antkowiak 1999). Third, propofol does not influence glutamatergic excitatory transmission (Bickler et al. 1995; Kitamura et al. 2003); this last point enabled us to simplify the analysis of the possible mechanisms involved.
Synaptic summation was evoked at relatively depolarized membrane potentials, so that repetitive synaptic stimuli could summate to fire single spikes, and low threshold conductances could be minimized (Pedroarena and Llinás 2001). Five synaptic stimuli evoked a single spike at the 5th stimulus when the membrane potential was held at -61 mV in control (Fig. 4Bi); bath-application of propofol (3 µM) increased the number of evoked spikes (Fig. 4Bii). Similar synaptic responses were evoked in another neuron (Fig. 4Ci), and bath application of apamin (100 nM) produced a response identical to that elicited by propofol (Fig. 4Cii); subsequent addition of propofol produced a similar response (Fig. 4Diii). The same responses were observed in 5/5 neurons. Again, this indicated that the effect of propofol was occluded by apamin, and that blockade of the SK channel during propofol application could enhance synaptic responses in RTN neurons.

**Propofol enhances successful GABAergic transmission at RTN-VB synapses**

As shown above, propofol enhanced GABAergic RTN neuron excitability and spike firing; we predicted that there should be a concomitant increase in GABAergic input to VB, and this possibility was directly investigated, using voltage-clamp recordings in VB neurons. We first examined whether the change of RTN excitability could alter the success rate of GABAergic synaptic transmission from RTN to VB (Ulrich and Huguenard 1995). GABA_A IPSCs were evoked in VB neurons clamped at -60 mV in the presence of 2-OH saclofen (100 µM) by extracellular stimulation of RTN, with stimulus intensity (20 – 100 µA, 0.15 ms, every 10 s) set to evoke EPSCs in ~50% of the stimulations in control (Mansvelder et al. 2002). Evoked fast unitary IPSCs (eIPSCs) had an average latency of 2.4 ± 0.8 ms (from time of stimulus to onset of IPSC). As illustrated in Fig. 5Ai, 10 synaptic stimuli evoked an average of 5 IPSCs (averaged
from 3 trials, with 10 stimuli/each). Bath-application of propofol (0.6 µM) increased the average number of evoked IPSCs to 8 (Fig. 5Aii) in the same neuron (using the same stimulation protocol). Normalized group data (Fig. 5Aiii) demonstrated that propofol increased the success rate of GABAergic transmissions at RTN-VB synapses in a concentration-dependent manner, strongly indicating a presynaptic site of action (Ulrich and Huguenard 1995).

Presynaptic mechanisms underlying the effect of propofol on GABAergic transmission may involve modulation of transmitter release, which is reflected by a change in spontaneous IPSC (sIPSC) frequency (Richards 2002). Spontaneous IPSCs (also termed “total” IPSCs) include action potential-dependent and -independent events; the two IPSC types can be distinguished by the use of tetrodotoxin (Nishikawa and MacIver 2000; Semyanov and Kullmann 2001). Spontaneous IPSCs were recorded in VB neurons at a holding potential of -60 mV in the absence of TTX (Fig. 5Bi), and bath application of propofol (0.6 µM) increased the frequency of total IPSCs (Fig. 5B); 3 µM-propofol produced a larger effect (traces not shown). Analysis of pooled data demonstrated that there was a significant difference in the frequency of total IPSCs before and during propofol application at 0.6 and 3 µM (Fig. 5Biv, P < 0.05, one way ANOVA, vs. control, n = 15).

Action potential-dependent IPSCs have been successfully used to study GABA release due to presynaptic spike firing (Dumoulin et al. 2001; Nishikawa and MacIver 2000), rather than the release machinery located in nerve terminals (Jarvis and Zamponi 2001; Dunlap et al. 1995; Richards, 2002). Presumed action potential-dependent IPSCs were defined as IPSCs > 240 pA (also termed large IPSCs), as under our experimental conditions no IPSC greater than this value was observed after application of tetrodotoxin (500 nM), which completely abolished action potential firing (not shown). Propofol at 0.6 and 3 µM markedly increased the large IPSC
frequency (P < 0.05, one way ANOVA, vs. control, Fig. 5Ci), but did not significantly increase large IPSC amplitude (Fig. 5Cii). Therefore, the data demonstrated that increasing RTN neuronal excitability was coupled to an increase in spontaneous IPSC frequency.

**Effects of propofol on miniature IPSCs**

Some anesthetics may alter GABA release by directly modifying the release machinery in nerve terminals (reviewed by Richards 2002). Such an effect can manifest itself by changing miniature IPSC (mIPSC) frequency, and this possibility was also tested in another subgroup of VB neurons. mIPSCs were recorded in VB neurons in the presence of TTX (500 nM) at a holding potential of -60 mV, and had an average amplitude of 43.2 ± 2 pA (n = 15; Fig. 6Ai). Bath-application of propofol significantly increased mIPSC amplitude by 36.1 ± 2.1% (58.8 ± 3 pA) and 44.7 ± 3.1% (62.6 ± 3.2 pA), at concentrations of 0.6 µM and 3 µM, respectively, (P < 0.05, one way ANOVA vs. control, n = 15, Fig. 6Bi, traces for 0.6 µM not shown). At the lower concentration (0.6 µM), propofol had little effect on mIPSC frequency. At the higher concentration (3 µM), however, propofol significantly increased frequency (19 ± 1.1%, from 13.7 ± 1.2 Hz to 16.3 ± 1.4 Hz, P < 0.05, one way ANOVA, vs. control, Fig. 6Bii). Since mIPSC amplitude was markedly increased by 3 µM-propofol, a moderate increase in the frequency might be due to an increase in event detection. To directly test to what extent mIPSC amplitude could affect the event detection probability, holding potentials were set to more positive levels so as to increase amplitude by a magnitude similar to that produced by propofol. At a holding potential of -50 mV (no propofol), mIPSC amplitude increased by 27.1 ± 2.2% (P < 0.05, compared to mIPSC amplitude recorded at -60 mV), but frequency did not change. At a holding potential of -40 mV, mIPSC amplitude was further increased (by 45.5 ± 3.5% compared to
mIPSC amplitude recorded at -60 mV) and mIPSC frequency was significantly increased (by 50.1%). The increase in mIPSC amplitude produced by the more positive holding potential (-40 mV) was essentially identical to that produced by 3 µM-propofol (45.4% and 44.7%, respectively). This indicated that a large increase in mIPSC amplitude could increase the probability of event detection, and that 3 µM-propofol might not directly modulate GABAergic terminals as to increase mIPSC frequency.

**Propofol enhanced GABA_A synaptic inhibition in thalamocortical neurons**

Clinical studies have shown that propofol-induced unconsciousness is accompanied by thalamic inhibition of somatosensory evoked responses in humans (Bonhomme et al. 2001). This inhibition may result from anesthetic potentiation of RTN-mediated inhibitory input to thalamocortical neurons. Therefore, the effect of propofol on synaptic inhibition of VB neuron spike firing was investigated. Tonic spike firing was initiated prior to stimulation (Fig. 7Ai). GABA_A IPSPs were evoked by extracellular single stimuli (50-100 µA, 0.15 ms, every 10 s) in the presence of 2-OH saclofen (100 µM). RTN stimulation produced a small IPSP (~5 mV in amplitude, ~60 ms in duration), and decreased spike firing (Fig. 7Aii). Evoked IPSPs and inhibition of spike firing could be reversibly blocked by the specific GABA_A receptor antagonist gabazine (10 µM, Fig. 7Aiii). Bath application of propofol (0.6 µM) during RTN stimulation increased IPSP amplitude (~9 mV) and prolonged the duration (~106 ms), thus enhancing the synaptic inhibition (Fig. 7Av). The propofol-potentiated IPSP and -enhanced inhibition of spike firing could be blocked by gabazine (Fig. 7Avi). A comparison of GABA_A IPSPs in the absence and presence of propofol is shown in Fig. 7Avii. Propofol potentiated synaptic inhibition of VB
neuron spike firing in a concentration-dependent manner (Fig. 7B). The concentration-effect curve was fit with the Hill function, yielding an estimated EC50 value of 0.5 µM.

We also examined whether propofol might have effects on depolarizing GABAA IPSPs or Cl⁻ reversal potential, since depolarizing IPSPs can directly trigger low-threshold spikes (Bazhenov et al. 1999). Both hyperpolarizing and depolarizing GABAA IPSPs were evoked with single stimuli in the presence of CGP55845 (1 µM) at various holding potentials (from -84 to -44 mV, see sample traces in Fig. 7Ci); bath application of propofol (0.6 µM) potentiated the synaptic response (Fig. 7Cii). Group data were fit with a linear equation, yielding a mean reversal potential of -71.1 and -70.7 mV in the control and propofol groups, respectively (Fig. 7Ciii). The values were close to calculated reversal potential of -69 mV for Cl⁻ (based on solutions for current clamp), and there was no significant difference in the mean reversal potential before and during propofol application. Propofol failed to alter the reversal potential or to trigger spike bursting from depolarizing IPSPs.

**RTN input is critical in mediating propofol’s effects in VB**

Next, we investigated the extent to which RTN-mediated inhibitory input influenced spike firing in VB neurons during propofol application. RTN is the sole source of GABAergic input to VB as the rodent VB does not contain GABAergic interneurons (Arcelli et al. 1997). In horizontally cut brain slices, RTN looks like a narrow band, lying anteriorly and laterally to VB, and a large part of the anterior (somatosensory sector) RTN projects to VB (Cox et al. 1996; Ohara and Lieberman 1985). This unique structure enabled us to minimize GABAergic input to VB by excising RTN and neighboring structures (e.g. the striatum, cortex, and other structures,
see Fig. 8A), thereby creating “RTN-removed” slices. “RTN-retained” slices were prepared from the contralateral hemisphere at an identical plane, and served as controls.

To test the extent to which GABAergic input to VB was altered in RTN-removed slices, we recorded sIPSCs and analyzed sIPSC frequency. In RTN-retained slices, there were robust sIPSCs (Fig. 8B, top panel), with a frequency of 18.2 ± 1.8 Hz and amplitude of 8 to 1500 pA. In RTN-removed slices, spontaneous IPSC frequency was reduced to 4.5 ± 1.2 Hz, and amplitude was much smaller (7 - 80 pA; Fig. 8C). The differences in sIPSC frequency and amplitude between RTN-removed and RTN-retained slices were significant (P < 0.001, t-test, n = 12). All IPSCs could be abolished by bicuculline (10 µM, not shown). The above data demonstrated that GABAergic input to VB was dramatically reduced following RTN excision.

Comparison of propofol-mediated suppression in VB neurons in RTN-retained and RTN-removed slices

As shown above, removal of RTN markedly reduced spontaneous IPSC frequency, suggesting that there was a parallel decrease in GABA release. A decrease in receptor-bound GABA should attenuate the effects of GABA_A receptor allosteric modulators such as propofol. Therefore, we compared the effect of propofol in RTN-removed and RTN-retained slices.

Single spike firing was initiated in a VB neuron in an RTN-retained slice (Fig. 9A, left), and bath application of propofol (1 µM) markedly decreased the number of spikes (right). In an RTN-removed slice, however, propofol suppression of spike firing was markedly attenuated (Fig. 9B). At a low concentration (0.6 µM) propofol had little effect in RTN-removed slices (Fig. 9C). Propofol decreased spike firing in a concentration-dependent manner. Concentration-effect curves were fit with the Hill function (Fig. 9D), yielding an estimated EC_{50} of 1.1 ± 0.3 (n
= 40) and 3.5 + 0.7 µM (n = 28) for RTN-retained and RTN-removed slices, respectively. There was a significant difference in the EC₅₀’s between RTN-retained and RTN-removed slices. The data clearly indicate that RTN is a critical structure for mediating propofol’s effects in thalamic circuits.

**GABA<sub>A</sub>-receptors underlie the shunting effects of propofol in postsynaptic VB neurons**

Since the GABAergic anesthetic pentobarbital can decrease input resistance via a non-GABAergic mechanism (Wan and Puil 2002), we examined mechanisms underlying the propofol-induced decrease in intrinsic excitability in VB neurons in RTN-retained slices. Input resistance in the absence of drug was 254 ± 8 MΩ (n = 90) in VB neurons. Bath application of propofol alone (1 - 10 µM) for 30 min suppressed spike firing (not shown), and decreased input resistance in a concentration-dependent manner (Fig. 10A), although low concentrations (< 3 µM) did not significantly affect input resistance. Normalized data are shown in Fig. 10A.

In another set of experiments, various compounds were tested to establish the mechanism for the propofol-induced decrease in input resistance. Propofol (6 µM) alone reduced input resistance in VB neurons (n = 15). Pre-application of the GABA<sub>A</sub> receptor antagonist bicuculline (10 µM, n = 15) did not significantly change input resistance, and subsequent co-application of propofol failed to affect input resistance (Fig. 10B). In a different group of neurons, pre-application of the GABA<sub>B</sub> receptor antagonist 2-OH saclofen (100 µM, n = 10) had no effect, and addition of propofol decreased input resistance by nearly the same amount as propofol alone. Brief focal application (200 ms) of the GABA<sub>A</sub> receptor agonist muscimol (1 µM in pipette) dramatically decreased input resistance and firing rate (traces not shown) in another 10 neurons, and this effect was completely reversed by addition of bicuculline. Finally,
blockade of a persistent Na\(^+\) conductance by tetrodotoxin (1 \(\mu\)M) had no significant effect on input resistance in another 10 neurons, and addition of propofol (6 \(\mu\)M) significantly reduced input resistance during hyperpolarization or depolarization induced by current pulses (-60 and 60 pA). These data clearly indicated that the GABA\(_A\) receptor-mediated Cl\(^-\) conductance produced shunting inhibition in VB neurons during propofol application, and this mechanism is distinct from that of pentobarbital (Wan and Puil 2002).
DISCUSSION

The present study demonstrated that propofol was capable of enhancing intrinsic excitability, temporal summation, and spike firing in GABAergic RTN neurons. The enhanced GABAergic inhibitory input resulted in shunting inhibition in postsynaptic glutamatergic VB neurons. We also demonstrated that thalamocortical suppression by propofol at clinically relevant concentrations critically depended on the presence and activation of the RTN. The observed contrasting responses to propofol in RTN and VB neurons appear consistent with the *in vivo* demonstration that propofol increases *c-fos* expression in a GABAergic nucleus and decreases its expression in a non-GABAergic nucleus in a sleep-promoting pathway (Nelson et al. 2002).

**Propofol inhibition of SK channels enhanced RTN neuron excitability**

Our study mainly focused on how propofol influenced intrinsic excitability in RTN neurons by testing changes in intrinsic responses (*i.e.*, input resistance, temporal summation of subthreshold membrane voltage, and spike firing). In hippocampal neurons, increasing input resistance increases temporal summation at the soma (Magee 1998; 1999). Here, we showed that propofol markedly increased apparent input resistance (Fig. 1 and 2), which prolonged the decay time of intrinsic subthreshold voltage responses in RTN neurons (Table 1). Correspondingly, propofol increased temporal summation of subthreshold voltage responses. This effect was also mimicked and occluded by the specific small conductance Ca$^{2+}$-activated K$^+$ (SK) channel blocker apamin.

The SK channel-mediated conductance is known to generate mAHP (Sah 1996; Stocker et al. 1999), and regulate neuronal excitability and tonic spike firing rate (Sah 1996; Stackman et al. 2002; Stocker et al. 1999; Stocker 2004). The SK2 channel protein is densely expressed in
RTN but not in VB (Sailer et al. 2002; Stocker and Pedarzani 2000). Thus, blockade of the SK channels during propofol application likely accounted for increased intrinsic excitability and spike firing rate in RTN neurons (Figs. 1 - 3), and this conclusion is consistent with previous findings that apamin-blockade of AHPs increases RTN neuron excitability (Bal and McCormick 1993; Debarbieux et al. 1998). The failure of propofol to affect fast-AHP in RTN is consistent with the insensitivity of the underlying potassium channel subtype to the low concentrations of propofol used here (Friederich and Urban 1999; Yamakura et al. 2001).

The SK channel-mediated conductance not only contributes to intrinsic excitability, but also regulates synaptic efficacy during synaptic stimulation through the modulation of postsynaptic cells (Borde et al. 1999). We found that propofol markedly enhanced EPSP summation in response to corticothalamic pathway stimulation (Fig. 4A and B). This enhancement was also primarily due to blockade of the SK channel in RTN neurons (Fig. 4C), but not due to a direct effect on glutamatergic axon terminals arising from corticothalamic neurons in the cortex, because low concentration-propofol (< 10 µM) does not increase glutamatergic transmission (Bickler et al. 1995; Buggy et al. 2000; Kitamura et al. 2003; Wakasugi et al. 1999; see also Wei et al. 2002). Our data support the conclusion that increased intrinsic excitability can increase synaptic responsiveness in thalamic neurons (Pedroarena and Llinás 2001).

Enhancement of GABAergic input is primarily mediated through an increase in RTN neuronal excitability

Changes in RTN neuron spike firing can also be detected by measuring spontaneous IPSCs in postsynaptic VB neurons. Propofol (0.6 µM) markedly increased the frequency of total spontaneous IPSCs (both action potential dependent- and independent-events). The increase in
IPSC frequency strongly suggests that propofol increased GABA release in VB. The question arises as to whether the increase in GABA release resulted from increased fiber excitability, direct modification of the axon terminal release machinery, or both.

To define the underlying mechanism, mIPSC frequency was measured in an effort to detect changes in transmitter release probability from axon terminals (Bennett and Kearns 2000; Stevens 1993; Vautrin and Barker 2003). Low concentration (0.6 µM)-propofol did not change mIPSC frequency, although it significantly increased mIPSC amplitude (which may improve event detection). High-concentration (3 µM) propofol significantly increased mIPSC frequency, and the same increase could be mimicked by increasing electromotive driving force (Fig. 6Cii). The data suggest that direct modulation of GABAergic terminals, if any, is not a major mechanism underlying GABA release during propofol application. Since propofol (0.6 µM) significantly increased both GABAergic synaptic transmission at RTN-VB synapses (Fig. 5A) and the frequency of large IPSCs (Fig. 5C), we conclude that low concentration-propofol increases the spontaneous IPSC frequency (or GABA release) by increasing RTN neuron spike firing rate (Figs. 1 and 2). This conclusion is consistent with other observations showing that low concentrations of propofol have little effect on the processes governing transmitter release from axon terminals (Mantz et al. 1995; Olcese et al. 1994; Shirasake et al. 2004; Takei et al. 2003; Westphalen and Hemmings 2003; Ya Deau et al. 2003; reviewed by Richards 2002).

**RTN input is important for mediating the effects of propofol in thalamic circuits**

We also demonstrated that propofol suppression of postsynaptic VB neurons depends on GABAergic input. Given that action potential-dependent GABA release from RTN neurons is required for GABAergic inhibition in VB neurons, removal of the RTN should attenuate such inhibition. Indeed, removing the RTN completely abolished the suppression elicited at low
concentrations (< 1 µM), and decreased the overall potency of propofol by 218% (i.e., increasing EC₅₀ from 1.1 to 3.5 µM). Therefore, the presence of the RTN is critical for propofol to elicit thalamocortical suppression at clinically relevant concentrations. As concentrations increased, propofol continued to suppress VB neurons in RTN-removed slices (Fig. 9D). This is likely due to residual GABA release from GABAergic terminals remaining in VB, and possibly direct activation of GABAₐ receptors by propofol (Sanna et al. 1995). Several sets of intrinsic conductances (e.g., Iₜ, Iₚ) are known to regulate VB neuron firing (Steriade et al. 1997), and the effect of propofol on these conductances warrants further study.

**Postsynaptic effects in VB neurons are mediated by GABAₐ receptors**

As shown in Figs. 7 and 9, propofol suppressed tonic spike firing in VB neurons. This suppression was associated with a decrease in apparent input resistance in VB neurons (Fig. 10A). The shunting inhibition, mimicked by the GABAₐ receptor agonist muscimol, could be blocked by the antagonism of GABAₐ, but not GABA₃, receptors. These data indicate that the postsynaptic shunting inhibition in glutamatergic VB neurons resulted from a propofol-mediated increase in the GABAₐ receptor-mediated chloride conductance, and is comparable to the effect seen in hippocampal neurons (Orser et al. 1994).

In summary, propofol activation of RTN neurons via blockade of SK channels represents a novel presynaptic mechanism of action. As a result of RTN activation, GABAergic transmission increased at RTN-VB synapses, and this increased the postsynaptic GABAₐ conductance in VB neurons, thereby “gating” thalamocortical spike output (Le Masson et al. 2002). Our results support the hypothesis that thalamocortical circuits are an important target for the general anesthetic propofol (Bonhomme et al. 2001; Fiset et al. 1999; Hofbauer et al. 2004; Menon 2001).
Acknowledgements:  Funding for this work was provided by the Dept. of Anesthesiology, WMC, and by NIH grant GM66840 (to PAG).
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Table 1. Effects of propofol on integrative properties of intrinsic subthreshold voltage responses and CT-evoked EPSPs in RTN neurons.

<table>
<thead>
<tr>
<th></th>
<th>Slope (mV/ms)</th>
<th>½ width (ms)</th>
<th>Decay time (ms)</th>
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<tbody>
<tr>
<td><strong>Intrinsic subthreshold voltage response</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>4.1 ± 0.8</td>
<td>24.2 ± 1.5</td>
<td>31.5 ± 1.8</td>
</tr>
<tr>
<td>propofol</td>
<td>4.4 ± 1.2</td>
<td>34.2 ± 3.6**</td>
<td>45.1 ± 4.1*</td>
</tr>
<tr>
<td><strong>Corticothalamic-evoked EPSP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>4.5 ± 0.8</td>
<td>28.2 ± 1.5</td>
<td>38.5 ± 1.8</td>
</tr>
<tr>
<td>propofol</td>
<td>4.8 ± 1.2</td>
<td>46.2 ± 3.6**</td>
<td>55.1 ± 4.1*</td>
</tr>
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</table>

Intrinsic subthreshold voltage responses were initiated by intracellular injection of a train containing 5 exponential current pulses (33 Hz, 200 pA), while EPSPs were evoked by extracellular stimulation of CT pathway (a train of 5 pulses, 33 Hz, 0.15 ms). Data are derived from experiments as described in Fig. 5. Slope (dv/dt) representing depolarization rate is measured from the rising phase (20-80%) of the first responses. ½ width is measured at 50% of peak amplitude; decay time (20-80%) representing repolarization rate is measured from the falling phase of the fifth response. * and **, P < 0.05 and P < 0.001, t-test, vs control.
FIG. 1. Propofol increases intrinsic excitability and spike firing in RTN neurons.

A: representative traces showing distinct voltage responses in RTN and VB neurons. In response to a hyperpolarizing current pulse (-500, 500 ms), a RTN neuron generally showed a shallow sag, with multiple rebound spike bursts and without ADP, as compared to prominent sag and ADP in a VB neuron. Bi: tonic spike firing was initiated in a different neuron with DC injection, and bath application of propofol (3 µM) increased spike firing (ii). Bi: bar graph summarizing effects of propofol on tonic spike firing rate. *, P < 0.05, one-way ANOVA, vs. control, n = 18.

Ci: intrinsic spike firing was initiated by intracellular current pulse injection (-400 and 100 pA, 500 ms). Cii: focal propofol (3 µM in pipette) increased tonic and rebound spike firing. Focal drug application was started 10 s prior to recordings. Ciii: overlay showing small voltage responses to a hyperpolarizing current pulse (-60 pA, 500 ms) in the absence and presence of propofol. Note that propofol increased input resistance. Civi: bar graph summarizing effects of propofol on input resistance. *, P < 0.05, one-way ANOVA, vs. control, n = 20. Note that amplitude scale for Ciii is different. Amplitude scale: 50 mV for Ci and Cii; 10 mV for Ciii.

The value to the left of each voltage trace in this and following figures indicates the membrane potential.

FIG. 2. Propofol suppresses medium AHPs but not fast AHPs in RTN neurons.

A: tonic spike firing was recorded from a RTN neuron (control). Focal application of propofol (3 µM in pipette) increased the firing frequency. B: comparison of the fast afterhyperpolarization potential (fAHP; left panel) and medium afterhyperpolarization potential (mAHP; right panel) in the absence (control) and presence of propofol. Note that propofol
markedly reduced mAHP but not fAHP (same neuron as in A). Each trace is an average of 500 successive spikes, and spikes are truncated to better view AHPs. Time scale: 5 ms for left and 100 ms for right panel. C: tonic spike firing was recorded from another RTN neuron (control). Focal application of the SK channel blocker apamin (200 nM in pipette) increased spike firing (middle panel), and addition of propofol (by bath) produced a similar effect (right panel). D: overlay of traces showing fAHP (Di) and mAHP (Dii) in the absence (control) and presence of apamin. Note that apamin markedly reduced mAHP but not fAHP. Diii: overlay of traces showing mAHP in the presence of apamin alone or apamin plus propofol. Note that apamin completely occluded the effect of propofol on mAHP. Each trace is an average of 500 successive spikes. Time scale: 2 ms for (Di), 100 ms for (Dii), and 50 ms for (Diii). E: bar graph summarizing the effects of propofol and apamin on mAHPs. *, P < 0.05, vs. control, one-way ANOVA with Tukey pair-wise comparisons, n = 15. F: bar graph summarizing the effects of apamin and propofol on spike firing; *, P < 0.05, vs. control, n = 14, the same statistical analyses here (and in G) as in E. G: bar graph summarizing the effect of apamin and apamin plus propofol on input resistance; the pooled data indicate that apamin increased input resistance. *, P < 0.05, vs. control. There was no significant difference in effects between propofol and apamin plus propofol or apamin and apamin plus propofol (E – G), indicating that apamin occluded effects of propofol on mAHP, spike firing and input resistance.

FIG. 3. Propofol enhances temporal summation of intrinsic subthreshold voltage responses via blockade of SK channels in RTN neurons.

Ai: intrinsic subthreshold voltage response was initiated in a RTN neuron with intracellular injection of an EPSC-shaped current train (33 Hz, 5 pulses, 200 pA, bottom). Aii: focal
application of propofol (3 µM in pipette) enhanced temporal summation. *Ai**: overlay of (i) and (ii) for comparison (1st and 5th responses indicated by arrows). *Aiv*: bar graph showing that propofol enhanced summation of intrinsic subthreshold responses in RTN neurons. *, P < 0.001, t-test, n = 15. *Bi*: in a different RTN neuron, the intrinsic voltage response was initiated with the same current pulse train as in Ai at more depolarized membrane potential. *Bii*: bath application of propofol increased the number of spikes. *Ci*: in another RTN neuron, focal application of apamin (200 nM in pipette) mimicked the effect of propofol. *Ci**: addition of propofol failed to alter spike generation, indicating that apamin occluded propofol’s effect.

**FIG. 4. Propofol-enhanced synaptic temporal summation is SK channel-dependent in RTN neurons.**

*Ai*: EPSPs were evoked in an RTN neuron by extracellular stimulation of corticothalamic axons (pulse duration = 0.15 ms, 5 pulses, 33 Hz). *Aii*: bath-application of propofol (3 µM) increased synaptic summation. *Ai**: overlay of (i) and (ii) for comparison of the voltage response. *Ai**: bar graph showing the effect of propofol on temporal summation of evoked EPSPs in RTN neurons. *, P < 0.001, t-test, n = 15. *Bi*: synaptic temporal summation was obtained at a more depolarized membrane potential in a different RTN neuron, culminating in single spike firing on 5th EPSP. *Bii*: propofol (3 µM) enhanced synaptic summation. *Ci*: in a different RTN neuron, synaptic temporal summation was obtained; *Ciii*: synaptic summation was enhanced by focal apamin (200 nM in pipette). *Ci**: focal apamin occluded the effects of propofol.
FIG. 5. Propofol enhances GABAergic transmission at RTN-VB synapses.

Ai: IPSCs were evoked in a VB neuron clamped at -60 mV by RTN stimulation with stimulus intensity (20 µA - 100, 150 µs, every 15 s) set to produce ~50% success rate in control (left). Aii: bath application of propofol (0.6 µM; right) increased the number of synaptically evoked IPSCs in the same cell as in Ai. Aiii: bar graph summarizing that propofol enhanced success rate of synaptic transmission in a concentration-dependent manner. *, P < 0.05, vs. control, one-way ANOVA, n = 15. Bi: spontaneous IPSCs were recorded in another VB neuron at -60 mV in a TC slice. Bii: propofol (0.6 µM) increased IPSC frequency. Note a marked increase in large-amplitude IPSC frequency. Biii: time course plot for pooled data (n = 15) showing an increase in total sIPSC frequency in the presence of propofol. Biv: bar graph summarizing the effect of propofol on total IPSC frequency at two different concentrations. *, P < 0.05 vs. control (0 µM), n = 15. Ci: bar graph showing that propofol significantly increased the frequency of large IPSCs. *, P < 0.05 vs. control. Cii: bar graph showing that propofol had no significant effect on the amplitude of large IPSCs (same neurons as in Biv).

FIG. 6. Effects of propofol on miniature IPSCs in VB neurons.

Ai: miniature IPSCs (mIPSCs) were recorded in a VB neuron at -60 mV in the presence of TTX. Aii: bath application of propofol (3 µM) increased mIPSC frequency, amplitude and prolonged the decay time. Bi-ii: bar graphs summarizing the effect of propofol on mIPSC amplitude and frequency; note that propofol at 0.6 µM does not increase mIPSC frequency. *, P< 0.05 vs.
control (0 µM), n = 15.  

**FIG. 7. Propofol enhances RTN-mediated synaptic inhibition in VB neurons.**

**Ai:** tonic spike firing was initiated in a VB neuron in a horizontal slice.  **Aii:** RTN stimulation (single pulse, 0.1 Hz) in the presence of 2-OH-saclofen (100 µM) evoked an IPSP, and decreased the tonic spike firing. Arrowhead marks onset of stimulation.  **Aiii:** bath application of gabazine (10 µM) blocked the synaptic inhibition.  **Aiv:** washout of gabazine.  **Av:** bath application of propofol (0.6 µM) potentiated the synaptic inhibition.  **Avi:** gabazine blocked the synaptic inhibition potentiated by propofol.  **Avii:** overlay of the IPSPs shown in (i) and (v); note that propofol increased IPSP amplitude and duration.  **B:** group data showing that propofol potentiated RTN-mediated synaptic inhibition of VB neuron spike firing in a concentration-dependent manner. The concentration-effect curve was fit with the Hill equation, yielding an estimated EC₅₀ of 0.5 µM; n = 10 –12 /each point.  

**C:** both hyperpolarizing and depolarizing IPSPs were evoked in the presence of CGP55845 (1 µM) at various holding potentials (Vₕ). Representative traces showing GABAₐ IPSPs in the absence (**Ci**) and presence (**Ciι**) of propofol (0.6 µM). Arrows indicate reversal potentials.  **Ciι:** IPSP amplitude data (n = 15 cells) were plotted as a function of holding potentials, and fit with a linear equation, yielding a reversal potential of -71.1 and -70.7 mV for control (open circle) and propofol (filled circle), respectively.

**FIG. 8. Comparison of spontaneous IPSCs in VB neurons in RTN-retained and -removed slices.**
A: a photomicrograph of a horizontal live slice containing both VB and RTN (position marked by arrow). The solid line indicates the cut through the slice to remove RTN and other structures. 

B: a sample trace showing spontaneous IPSCs (sIPSCs) recorded from a RTN retained slice (top), and group data (bottom) showing the sIPSC frequency in such slices (n = 30). Error bars are omitted for clarity. 

C: a sample trace showing sIPSCs recorded from an RTN-removed slice (top; same scale as in B); group data (bottom) showing the sIPSC frequency in RTN-removed slices (n = 12). Note that the sIPSC frequency was dramatically reduced in RTN-removed slices. 

D: Cumulative amplitude distributions from pooled results for sIPSCs in RTN-retained and RTN-removed slices. The difference in the cumulative probability is significant between RTN-retained and removed slices (P < 0.05, Kolmogorov-Smirnov test). Data derived from B and C.

FIG. 9. Comparison of propofol’s effects in VB neurons in RTN-retained and -removed slices.

A: (left) sample trace showing tonic spike firing recorded from a VB neuron in a RTN retained slice. Spikes are truncated for clarity in all panels. (right) bath application of propofol decreased spike firing. 

B: (left) spike firing recorded from another VB neuron in an RTN-removed slice, and (right) inhibitory effects of propofol were markedly attenuated. 

C: group data showing that a significant inhibitory effect produced by a low concentration (0.6 µM) of propofol was observed only in RTN-retained slices, but not in RTN-removed slices. *, P < 0.05, one way ANOVA, vs. control, n = 12/each. 

D: concentration-effect curves for the inhibitory effects of propofol on VB neuron firing in RTN-retained and -removed slices. There is a significant difference in effects of propofol between RTN-retained and removed groups, P < 0.05, t-test, n = 12 - 15/each data point.
FIG. 10. Propofol decreases input resistance in VB neurons via GABA<sub>A</sub> receptors.

A: propofol decreased input resistance in a concentration-dependent manner. *, P < 0.05 vs. control, one-way repeated measures ANOVA (Bonferroni test); n is shown within the bar.  
B: group data demonstrating that shunting effects are mediated by GABA<sub>A</sub> receptor chloride channels, rather than other ion channels tested. *, P < 0.05 vs. control, one-way repeated measures ANOVA with Tukey’s post hoc test.  prop = propofol (6 µM), bic = bicuculline (10 µM), sacl = 2-OH saclofen (100 µM), mus = muscimol (1 µM), TTX = tetrodotoxin (1 µM).
FIG. 1. Propofol increases intrinsic excitability and spike firing in RTN neurons. (Ying & Goldstein, Fig width = 3.8 inches)
Fig. 2. Propofol suppresses apamin-sensitive mAHP in RTN neurons. (Ying & Goldstein, Fig width = 4.9 inches)
FIG. 3. Propofol enhances temporal summation of intrinsic subthreshold voltage responses via blockade of SK channels in RTN neurons
(Ying & Goldstein, Fig width = 4 inches)
FIG. 4. Propofol-enhanced synaptic temporal summation is SK channel-dependent in RTN neurons (Ying & Goldstein)
FIG. 5. Propofol enhances GABAergic transmission at RTN-VB synapses
(Ying & Goldstein, Fig width = 4.98 inches)
FIG. 6. Effects of propofol on mIPSCs in VB neurons (Ying & Goldstein)
FIG. 7. Propofol enhances RTN-mediated GABA<sub>A</sub> inhibition in VB neurons. (Ying & Goldstein, Fig width 4.2 = inches)
FIG. 8. Comparison of sIPSCs in VB in RTN-retained and RTN-removed slices (Ying & Goldstein, Fig width = 5.12 inches)
FIG. 9. Comparison of propofol's effects in VB neurons in RTN-retained and -removed slices
(Ying & Goldstein, Fig width = 5.12 inches)
FIG. 10. Propofol decreases input resistance in VB neurons via potentiation of postsynaptic GABA$_A$ receptors
(Ying & Goldstein, width 3.5 inches)