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# Disruption of Coherent Oscillations in Inhibitory Networks With Anesthetics: Role of GABA<sub>A</sub> Receptor Desensitization

PAMELA M. BAKER,<sup>1,3,4</sup> PETER S. PENNEFATHER,<sup>3,5</sup> BEVERLEY A. ORSER,<sup>3,6</sup>  
AND FRANCES K. SKINNER<sup>1–4</sup>

<sup>1</sup>Toronto Western Research Institute, University Health Network, <sup>2</sup>Department of Medicine (Neurology), <sup>3</sup>Department of Physiology and <sup>4</sup>Institute of Biomaterials and Biomedical Engineering, <sup>5</sup>Departments of Pharmaceutical Sciences and Pharmacology, <sup>6</sup>Department of Anaesthesia and Sunnybrook and Women's College Health Sciences Center, University of Toronto, Toronto, Ontario M5T 2S8, Canada

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**Baker, Pamela M., Peter S. Pennefather, Beverley A. Orser, and Frances K. Skinner.** Disruption of coherent oscillations in inhibitory networks with anesthetics: role of GABA<sub>A</sub> receptor desensitization. *J Neurophysiol* 88: 2821–2833, 2002; 10.1152/jn.00052.2002. The effect of anesthetic drugs at central synapses can be described quantitatively by developing kinetic models of ligand-gated ion channels. Experiments have shown that the hypnotic propofol and the sedative benzodiazepine midazolam have similar effects on single inhibitory postsynaptic potentials (IPSPs) but very different effects on slow desensitization that are not revealed by examining single responses. Synchronous oscillatory activity in networks of interneurons connected by inhibitory synapses has been implicated in many hippocampal functions, and differences in the kinetics of the GABAergic response observed with anesthetics can affect this activity. Thus we have examined the effect of propofol and midazolam-enhanced IPSPs using mathematical models of self-inhibited one- and two-cell inhibitory networks. A detailed kinetic model of the GABA<sub>A</sub> channel incorporating receptor desensitization is used at synapses in our models. The most dramatic effect of propofol is the modulation of slow desensitization. This is only revealed when the network is driven at frequencies that are thought to be relevant to cognitive tasks performed in the hippocampus. The level of desensitization at synapses with propofol is significantly reduced compared to control synapses. In contrast, midazolam increases macroscopic desensitization at network synapses by altering receptor affinity without concurrently modifying desensitization rates. These differences in gating between the two drugs are shown to alter network activity in stereotypically different ways. Specifically, propofol dramatically increases the amount of excitatory drive necessary for synchronized behavior relative to control, which is not the case for midazolam. Moreover, the range of parameters for which synchrony occurs is larger for propofol but smaller for midazolam, relative to control. This is an important first step in linking alterations in channel kinetics with behavioral changes.

## INTRODUCTION

The use of anesthetic drugs in surgery is one of the most important advances in modern medicine, but the mechanism of action of many of these drugs remains elusive. There is growing evidence that most anesthetics act on the CNS through direct and specific interactions with a myriad of molecular

targets (Franks and Lieb 1998), including the GABA<sub>A</sub> receptor (Hirota et al. 1998; Tanelian et al. 1993). What is not known is how these interactions at the molecular and cellular level might give rise to the reductions in perception and cognition of subjects under anesthesia. Of particular interest is the phenomenon of receptor desensitization because different classes of anesthetics differ in their influence on this aspect of channel gating. Experiments in cultured hippocampal neurons of currents evoked by exogenous GABA show that the hypnotic drug propofol can potentiate inhibition by reducing the rate of onset of desensitization and slowing the rate of deactivation of the GABA<sub>A</sub> channel (Bai et al. 1999; Orser et al. 1994). In comparison, the sedative benzodiazepine midazolam slows the deactivation of hippocampal GABA<sub>A</sub> receptors; however it has no concurrent effect on the kinetics of slow desensitization (Ghansah and Weiss 1999; Orser and MacDonald 1996). These drugs have very similar effects on unitary synaptic responses but have significantly different behavioral effects. Propofol is used to cause a rapid loss of consciousness (or hypnosis) and can also be used alone as an anesthetic under certain conditions (Larijani et al. 1989). This is in contrast to midazolam, which acts as a sedative-amnestic drug. To define mechanisms of anesthesia, it is critical to explain how anesthetics might alter dynamic behavior of neuronal networks and systems, because it is these dynamics that ultimately determine behavior. Here we propose a strategy for translating the influence of anesthetics on channel gating, in particular receptor desensitization, to changes in neuronal network activity. This is an important first step in linking alterations in channel kinetics with behavioral changes.

Networks of inhibitory neurons connected by GABAergic synapses have been proposed to serve an important function for information processing in various cortical regions (Buzsáki and Chrobak 1995; Tamas et al. 1998). Specifically, oscillatory activity in the hippocampus in the theta (8–12 Hz) and gamma (20–80 Hz) bands has been implicated in cognitive tasks such as memory formation and spatial navigation (Bragin et al. 1995; Chrobak and Buzsáki 1998; Lisman and Idiart 1995). Experimental studies suggest that these oscillations arise from synchronous activity in inhibitory interneuronal networks (Traub et al. 1996; Whittington et al. 1995), and this activity can be disrupted by propofol but not midazolam (Faulkner et

Address for reprint requests: F. K. Skinner, Toronto Western Research Institute, University Health Network, 399 Bathurst St., MP 13-317, Toronto, Ontario, Canada, M5T 2S8 (E-mail: fskinner@uhnres.utoronto.ca).

al. 1998; Whittington et al. 1996). Receptor desensitization could play an important role in shaping network activity because desensitization with a slow time course of recovery not only reduces the amplitude of responses to agonist application but also prolongs synaptic responses (Bai et al. 1999; Jones and Westbrook 1995). Changes in the duration of synaptic responses have important consequences for inhibitory network behavior because the rate of decay of synaptic responses can significantly alter firing frequency and the ability of the network to synchronize as shown in various theoretical studies of inhibitory network models (Wang and Buzsáki 1996; Wang and Rinzel 1992; White et al. 1998).

In this paper, we investigate how the changes in receptor kinetics at the synapse observed with propofol and midazolam drugs might support mechanisms that disrupt synchronized oscillations in inhibitory networks. Identification of such mechanisms may make modulation of activity through changes in the kinetics of desensitization at synapses in these networks plausible as a mechanism of anesthesia. A kinetic model of the synapse that incorporates a detailed description of the channel gating is used in the network models. We show that the different classes of anesthetics give rise to different network states that differ in their ability to support synchronous oscillations. The resulting differences in network dynamics may contribute to differences in the behavioral effects observed for the different drugs. By explicitly considering actions at the level of GABA<sub>A</sub> receptor dynamics in our network model, we provide an essential, initial step for translating between levels of organization in the CNS. This may offer a more complete understanding of the changes in cognitive function seen with these drugs.

METHODS

Hippocampal interneuron model

We model single interneurons using Hodgkin-Huxley type equations describing Na<sup>+</sup>, K<sup>+</sup> and leak currents, with parameters derived previously to reproduce the excitability of hippocampal CA1 interneurons (Wang and Buzsáki 1996). Although the model is minimal, it successfully preserves the relationship between firing frequency and injected current observed experimentally for these neurons. The model is a single compartment, with membrane voltage, V, described by the equation

$$C_m \frac{dV}{dt} = I_{app} - g_{Na} m^3 h (V - V_{Na}) - g_K n^4 (V - V_K) - g_{leak} (V - V_{leak}) - I_{syn} \quad (1)$$

with

$$m_\infty = \frac{\alpha_m}{\alpha_m + \beta_m}$$

$$\alpha_m(V) = \frac{-0.1(V + 35)}{\exp(-0.1(V + 35)) - 1}$$

$$\beta_m(V) = 4 \exp(-(V + 60)/18)$$

$$\frac{dh}{dt} = \phi(\alpha_h(1 - h) - \beta_h h)$$

$$\alpha_h(V) = 0.07 \exp(-(V + 58)/20)$$

$$\beta_h(V) = \frac{1}{\exp(-0.1(V + 28)) + 1}$$

$$\frac{dn}{dt} = \phi(\alpha_n(1 - n) - \beta_n n)$$

$$\alpha_n(V) = \frac{-0.01(V + 34)}{\exp(-0.1(V + 34)) - 1}$$

$$\beta_n(V) = 0.125 \exp(-(V + 44)/80)$$

where m and h are the sodium activation and inactivation variables, respectively, m<sub>∞</sub> is the steady-state sodium activation function, n is the potassium activation, t is time, I<sub>app</sub> is the applied excitatory input current, and I<sub>syn</sub> is the synaptic GABAergic current (see following section).

Parameters governing the intrinsic currents in the interneuron model are taken from Wang and Buzsáki (1996) and are not varied in our simulations

$$g_{Na} = 35 \frac{mS}{cm^2} \quad V_{Na} = 55 \text{ mV} \quad g_K = 9 \frac{mS}{cm^2}$$

$$V_K = -90 \text{ mV} \quad g_{leak} = 0.1 \frac{mS}{cm^2} \quad V_{leak} = -65 \text{ mV}$$

$$C_m = 1 \frac{\mu F}{cm^2} \quad V_{syn} = -75 \text{ mV} \quad \phi = 5$$

where C<sub>m</sub> is the membrane capacitance, g<sub>Na</sub>, g<sub>K</sub>, and g<sub>leak</sub> are the sodium, potassium and leak conductances, respectively, and V<sub>Na</sub>, V<sub>K</sub>, and V<sub>leak</sub> are the sodium, potassium and leak reversal potentials, respectively.

GABA<sub>A</sub> synapse model

To quantify the effects of propofol and midazolam on the kinetics of GABAergic responses, we incorporate a model of the GABA<sub>A</sub> receptor developed by Bai et al. (1999) at synapses in our model neurons (Fig. 1). The model incorporates three closed states [unbound (C), monoliganded (L<sub>1</sub>C) and doubly-ligand bound (L<sub>2</sub>C)], two desensitized states [1 with a fast recovery time constant (L<sub>2</sub>D<sub>f</sub>) and 1 with a much slower recovery (L<sub>2</sub>D<sub>s</sub>)] and one open conducting state

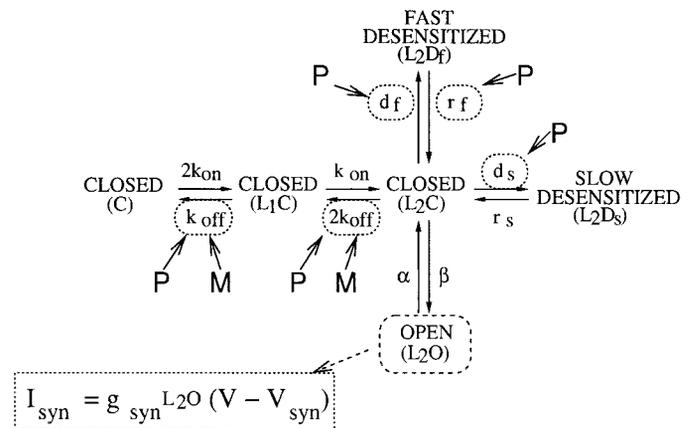


FIG. 1. A kinetic model of the GABA<sub>A</sub> receptor from Bai et al. (1999). It incorporates 2 ligand-bound closed states (L<sub>1</sub>C, L<sub>2</sub>C), 1 open (conducting) state (L<sub>2</sub>O), and 2 ligand-bound desensitized states: a fast component of desensitization (L<sub>2</sub>D<sub>f</sub>) that affects mainly the peak amplitude of the synaptic response and a more slowly developing component of desensitization (L<sub>2</sub>D<sub>s</sub>) that can reduce the amplitude of responses by up to 90% when the synapse is stimulated at high frequencies. The rate constants governing transitions between states were derived from experiments on cultured hippocampal neurons, and are given in Table 1. Propofol (P) and midazolam (M) affect particular rate constants as indicated. This kinetic model is linked to the interneuronal network model via the open, conducting state, L<sub>2</sub>O, affecting synaptic current, I<sub>syn</sub> as indicated. See text for details.

(L<sub>2</sub>O). The rate constants in the model are fit to GABAergic currents from hippocampal neurons in culture under control conditions and in the presence of propofol. In addition, we have modelled the effect of midazolam by switching the rate of deactivation ( $k_{\text{off}}$ ) of the receptor to that observed with propofol without changing the rates of desensitization (Table 1 and see Fig. 1). These effects of midazolam have also been described qualitatively in previous experiments (Ghansah and Weiss 1999; Orser et al. 1998). We have, however, increased the rate of recovery from slow desensitization ( $r_s$ ) from that published previously that was derived for a nucleated patch preparation (Bai et al. 1999). This rate change was necessary because the neuronal firing rate led to a greater than observed buildup of desensitization with the slower recovery rate under the stimulation conditions used. This faster recovery would be more consistent with the observed rate of recovery from a more intermediate state of desensitization that is observed with whole cell recordings (Orser et al. 1994). Although desensitization is still more pronounced than is typically observed in more intact preparations, we did not want to change more rate constants, especially those shown to be affected by propofol. Our aim here was not to generate a perfect simulation of the observed data. Rather this work represents an initial step in our attempt to understand how diverse effects of drugs on GABA channel kinetics can be linked to their well-known differences in behavioral effects.

The equations governing the synaptic variables in the interneuron model are derived from the kinetic scheme of the GABA<sub>A</sub> receptor (Fig. 1). Transitions between states in the scheme are first-order kinetic processes, and are described by the following differential equations

$$\frac{dC}{dt} = k_{\text{on}}L_1C - 2k'_{\text{on}}C$$

$$\frac{dL_1C}{dt} = 2k'_{\text{on}}C + 2k_{\text{off}}L_2C - (k_{\text{off}} + k'_{\text{on}})L_1C$$

$$\frac{dL_2C}{dt} = k'_{\text{on}}L_1C + \alpha L_2O + r_f L_2D_f + r_s L_2D_s - (\beta + d_f + d_s + 2k_{\text{off}})L_2C$$

$$\frac{dL_2O}{dt} = \beta L_2C - \alpha L_2O$$

$$\frac{dL_2D_f}{dt} = d_f L_2C - r_f L_2D_f$$

$$\frac{dL_2D_s}{dt} = d_s L_2C - r_s L_2D_s$$

where  $k'_{\text{on}} = F(V_{\text{pre}}) * k_{\text{on}} * \text{conc}$ ,  $F(V_{\text{pre}}) = 1 / \{1 + \exp[-(V_{\text{pre}} - \theta) / 2]\}$ ,  $\text{conc} = 0.003 \text{ M}$ ,  $\theta = 0 \text{ mV}$  and  $V_{\text{pre}}$  is the membrane voltage of the presynaptic cell.

Note that the ligand binding rate  $k'_{\text{on}}$  links the signal to the time course of GABA in the synaptic cleft. For convenience, we do this by modifying the rate  $k_{\text{on}}$  by a sigmoid function of presynaptic voltage,  $F(V_{\text{pre}})$ . This function is close to zero when the presynaptic neuron is

silent but quickly approaches one for the duration of the presynaptic action potential. This pulse approximates the rapid rise and fall that is characteristic of the temporal profile of neurotransmitter in the synaptic cleft and is concurrent with presynaptic activity (Destexhe et al. 1994). The parameter  $\text{conc}$  represents concentration of GABA in the synaptic cleft and is set to 3 mM (Bai et al. 1999; Clements 1996). The parameter  $\theta$  is the threshold for the sigmoid function, and sets the duration of the transmitter pulse in the cleft, which is approximately 0.5 ms at the value of  $\theta$  used in our simulations.

The proportion of the total population of receptors in a given state at a given time is equivalent to the probability of a single receptor being in that state at that time. Therefore, since the only variable in the kinetic scheme that represents an open (conducting) state is L<sub>2</sub>O, the equation for the synaptic current is

$$I_{\text{syn}} = \sum_{i=1}^N \frac{g_{\text{syn}}}{N} L_2O_i (V - V_{\text{syn}})$$

where  $N$  is the number of cells in the network (note that this includes self-inhibition) and  $g_{\text{syn}}$  is the maximal synaptic conductance, i.e., the conductance obtained if all GABAergic synapses on the neuron were activated simultaneously.

### Network simulations

In general, we focus on theta/gamma rhythmic frequencies that would broadly encompass 8–80-Hz frequencies because GABAergic network synchrony is associated with these rhythms and these frequencies are associated with higher cognitive processing (Buzsáki 2001). In particular, interconnected GABAergic fast-spiking interneurons (basket cells) in CA1 hippocampus can give rise to gamma oscillations (Wang and Buzsáki 1996). A summary of the various simulations performed in this paper are given in Table 2.

Simulations of single IPSPs are performed using a model consisting of two cells, one of which inhibits the other when stimulated with a brief pulse of injected current (amplitude = 10  $\mu\text{A}/\text{cm}^2$  and duration = 1 ms). The maximal conductance at the synapse connecting the two cells is set to our approximation for the conductance at a unitary synapse (see following text) and is 0.015  $\text{mS}/\text{cm}^2$ .

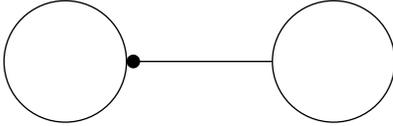
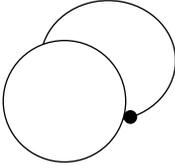
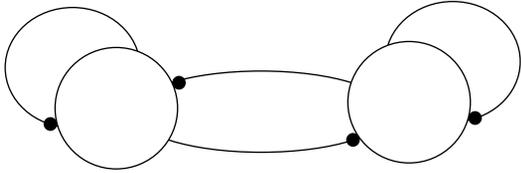
We investigate network frequency by performing simulations with an autaptic (self-inhibiting) single cell model. Such a single cell network model can be considered as a representative of a synchronously firing large population of homogeneous cells. Chow and colleagues (Chow et al. 1998; White et al. 1998) showed that this single self-inhibited cell gives insight into the coherence properties of larger heterogeneous networks. For these simulations, we set the maximal synaptic conductance  $g_{\text{syn}}$  and the applied excitatory input current  $I_{\text{app}}$  at values for which the model neuron fires at approximately 40 Hz (gamma range) for single-cell simulations when the synapse is allowed to reach an equilibrium level of desensitization ( $I_{\text{app}} = 1.25 \mu\text{A}/\text{cm}^2$ ,  $g_{\text{syn}} = 0.75 \text{ mS}/\text{cm}^2$ ).

We examine network frequency and correlation in the face of heterogeneity using a model with two cells that are both mutually and self-inhibitory. For these simulations, we allow  $g_{\text{syn}}$  and  $I_{\text{app}}$  to vary. The model should behave as close to actual physiological networks as possible. Therefore, we estimate the range of values studied for the maximal  $g_{\text{syn}}$  using anatomical data of synapses onto hippocampal interneurons (Gulyás et al. 1999; Nusser et al. 1998) and physiological data that describes IPSPs originating from interneurons (Buhl et al. 1995; Cobb et al. 1997). Briefly, we calculate  $g_{\text{syn}}$  using an estimate of the number of synaptic contacts within the proximal dendrites and soma of hippocampal basket cells from Gulyás et al. (1999), [508 boutons gives approximately 51 unitary (cell-cell) contacts], combined with the estimated conductance through a unitary synapse from

TABLE 1. Kinetic rates used at the synapse

Parameter	Rate Constants (/ms)		
	Control	With propofol	With midazolam
$k_{\text{on}}$	1000/M	1000/M	1000/M
$k_{\text{off}}$	0.103	0.056	0.056
$d_f$	3.0	1.62	3.0
$r_f$	0.2	0.12	0.2
$\alpha$	0.4	0.4	0.4
$\beta$	6.0	6.0	6.0
$d_s$	0.026	0.014	0.026
$r_s$	0.0001	0.0001	0.0001

TABLE 2. Summary of network simulations using GABA<sub>A</sub> receptor kinetic scheme (see Fig. 1)

Model Used	To Examine
 <p>Single IPSP</p>	IPSP amplitude and duration changes in a model interneuron (see Fig. 2)
 <p>Autapse (approximately equivalent to a large, homogeneous, synchronized network)</p>	IPSP amplitude and network frequency changes in a (physiologically relevant) rhythmically firing network (see Figs. 3, 4, 5, and 7)
 <p>Two-Cell (heterogeneous) Network</p>	Network frequency and correlation in a (physiologically relevant) rhythmically firing network (see Fig. 6)

IPSP, inhibitory postsynaptic potential.

Buhl et al. (1995) (1 nS). Additionally, we check this measure by using a second set of experimental data, taking an estimate of the number of receptors at an interneuronal synapse from Nusser et al. (1998) and multiplying by the single channel conductance through the GABA<sub>A</sub> channel (21 pS) and then assuming the same number of synapses as for the previous method (Gulyás et al. 1999). These estimates are then scaled according to the surface area of the soma and proximal dendrites of a hippocampal basket cell (7,400 μm<sup>2</sup> from Gulyás et al.), and a range of 0.4–1.3 mS/cm<sup>2</sup> is obtained. This estimate takes into account that the anatomical number of synapses may overestimate the number of functional synapses due to the observed high number of inactive synapses on hippocampal neurons (Kannenberget al. 1999). These  $g_{syn}$  values differ from those used previously in modelling studies (Wang and Buzsáki 1996) but are in agreement with more recent experimental data (Bartos et al. 2001).

For the two-cell network model, the cells are identical in their synaptic and intrinsic properties for each different simulation and differ only in the amount of excitatory drive,  $I_{app}$ , that they receive. This difference in excitation introduces heterogeneity into the network, allowing us to test the ability of neurons in the network to synchronize. The value of  $I_{app}$  for both cells in our simulations is chosen randomly for a given mean  $I_{app}$  by using a Box-Muller algorithm for generating Gaussian distributed numbers with standard deviation  $\sigma = 0.01$  for all of our runs. This value of  $\sigma$  is low enough so that synchronized behavior is still possible (Wang and Buzsáki 1996).

We integrate the resulting set of differential equations using the software package XPPAUT (G. B. Ermentrout, University of Pittsburgh, <http://www.math.pitt.edu/~bard/bardware/>) to obtain the single IPSP and autaptic model results in Figs. 2–5, and 7. For all other simulations we use the program PNNET, a version of the C++ program NNET developed in our lab (Skinner and Liu 2002) that has been modified to compute the multiple synaptic variables in our model. This program integrates differential equations using the program CVODE (Cohen and Hindmarsh 1996).

### Data analysis

The correlation or coherence measure we use to determine the level of synchrony between neurons for our two-cell simulations is taken from White et al. (1998). Briefly, both spike trains are approximated by a series of square pulses of unit height and fixed width of 40% (for Fig. 6) of the period of the fastest firing cell. Each square wave is centred around the peak of the individual action potentials in the train. The shared area of the square pulses from each train that overlap in time is then calculated for the duration of the simulation. This is equivalent to taking the cross-correlation of the two waves at zero time-lag. Thus the coherence measure, or more strictly, the correlation is calculated as the sum of the shared areas of all the pulses divided by the square root of the product of the total areas of each individual train of pulses; that is, if  $X(t)$  is the series of unit height pulses for the first cell over  $N$  time steps and  $Y(t)$  is the series of pulses for the second cell, then the coherence measure or correlation is calculated as

$$\text{Correlation (Coherence Measure)} = \frac{\sum_{t=1}^N X(t) * Y(t)}{\sqrt{\sum_{t=1}^N X(t) * \sum_{t=1}^N Y(t)}} \quad (2)$$

Any use of the term “coherent” throughout the manuscript refers to precisely synchronous and phase-locked activities as described in the preceding text.

### Calculation of the synaptic time constant

We calculate the synaptic time constants using the program Clampfit v8.0 (Axon Instruments, Union City, CA) for the single IPSPs in Fig. 2. We wish to compare the time constant of decay of our simulated IPSPs with values obtained for real neurons, so we fit the data to a standard exponential function using two terms as is common for experimental data. It should be noted that a better fit can be obtained using three terms with the additional term representing the time constant of fast desensitization.

## RESULTS

Modelling studies have identified several parameters that determine frequency and coherence in inhibitory networks. These are the maximal synaptic conductance,  $g_{\text{syn}}$  and synaptic time constant,  $\tau_{\text{syn}}$ , which shape the amplitude and duration of inhibitory responses, and the applied excitatory drive,  $I_{\text{app}}$ , which determines the firing frequency of the model neurons. White et al. (1998) show that inhibitory networks can be classified into two different regimes, the *phasic* or the *tonic* depending on the values that these parameters take in a given network. We exploit their analysis to explain differences in networks with or without drug. For our simulations, it is  $\tau_{\text{syn}}$  which is of particular interest because it is this value that is altered by addition of anesthetic. The other parameters,  $g_{\text{syn}}$  and  $I_{\text{app}}$ , are adjusted to match their physiological values (see METHODS).

We use three different model architectures to explore the properties of synapses and networks in the absence or presence of drug: a model of two cells connected by a unitary synapse without persistent excitatory drive, so that single IPSPs can be characterized; an autaptic cell to show how alterations in desensitization and deactivation at the synapse influence firing frequency in a homogeneous network; and a two-cell network that allows us to judge the ability of the network to synchronize when there is heterogeneity in the amount of input each cell receives (see Table 2). Using these three different model networks, we are able to give a mechanistic explanation of the differences between networks with and without the GABAergic drugs midazolam and propofol. The benzodiazepine midazolam is a sedative-amnestic drug that (in adults) does not produce a level of neurodepression needed to provide surgical anesthesia, while propofol is a hypnotic drug that can be used alone under certain conditions to mediate anesthesia.

*Effect of changes in rates altering desensitization and deactivation on amplitude and duration of IPSPs in the model interneuron*

Synaptic kinetics are critical for determining network behavior. Therefore first, we concentrate on how varying the rates in the kinetic scheme for the GABA<sub>A</sub> receptor that change upon addition of drug will affect amplitude and duration of the synaptic response. The effect of varying the different rates is predictable to some extent from the equation used to approximate the deactivation time constant from Bai et al. (1999) (see Eq. 1 in Bai et al. 1999). However the inhibitory responses in our cells are shaped not only by the time course of the synaptic variables but also the intrinsic properties of our interneuron model. We address this interaction by first simulating isolated IPSPs.

Figure 2 shows how amplitude and duration of single IPSPs are altered as rates of deactivation ( $k_{\text{off}}$ ) and desensitization ( $d_f$ ,  $r_f$ , and  $d_s$ ) vary. For these simulations,  $g_{\text{syn}}$  and  $I_{\text{app}}$  are held at a fixed value so that observed differences are solely attributable to changes in the kinetic rates. All receptors start in the closed, unbound state, i.e., all the variables representing the different kinetic states of the receptor are initially set to zero except for the ligand-unbound closed state (C) which is set to one. The IPSP is initiated when the post-synaptic cell is at its resting membrane potential ( $-64$  mV). Each of the rate constants that changes upon addition of drug is varied individually over a

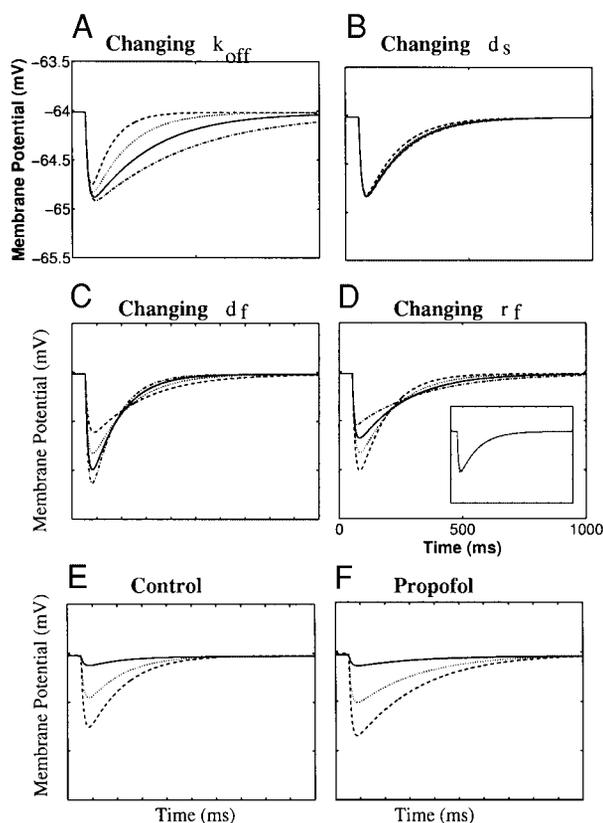


FIG. 2. Unitary inhibitory postsynaptic potentials (IPSPs) with the kinetic scheme from Bai et al. (1999). In A–D, the specified rate in the kinetic scheme for the GABA<sub>A</sub> receptor is varied individually and the rest of the rates remain at control values (all rates in units/ms): A:  $k_{\text{off}} = 0.2$  (---),  $0.103$  (···),  $0.056$  (—), and  $0.03$  (- · - ·). B:  $d_s = 0.050$ , (---),  $0.026$  (···),  $0.014$  (—),  $0.007$  (- · - ·). C:  $d_f = 6.0$  (---),  $3.0$  (···),  $1.62$  (—),  $0.8$  (- · - ·). D:  $r_f = 0.4$  (---),  $0.2$  (···),  $0.12$  (—),  $0.07$  (- · - ·); inset:  $d_f = 1.62$ ,  $r_f = 0.12$  as for propofol (---) and control (—). In the inset, note that the amplitude change is negligible. E and F: rates are held at control values (E) or propofol values (F) and IPSPs are plotted for initial conditions of the synaptic variables  $C = 0.9$ ,  $L_2D_s = 0.1$  (---);  $C = 0.5$ ,  $L_2D_s = 0.5$  (···); and  $C = 0.1$ ,  $L_2D_s = 0.9$  (—). For these simulations,  $g_{\text{syn}} = 0.015$  (mS/cm<sup>2</sup>). Note that x and y axes for A–F are all exactly the same as indicated.

range that encompasses both control and drug values. This approach isolates the contribution of each rate constant to the synaptic response and allows the magnitude of the effect of changing rates between drug and control values to be gauged. Changes in the dissociation rate constant,  $k_{\text{off}}$ , have the greatest effect on  $\tau_{\text{syn}}$ , varying from 79.2 ms at  $k_{\text{off}} = 0.20$ /ms to 399.0 ms when  $k_{\text{off}} = 0.030$ /ms.  $\tau_{\text{syn}}$  values of 145.2 and 245.8 ms are obtained when  $k_{\text{off}}$  takes control and drug values, respectively (Fig. 2A). Varying  $k_{\text{off}}$  has negligible effects on amplitude of the IPSP. In contrast, changing  $d_s$  has no significant influence on  $\tau_{\text{syn}}$ , varying from 158.5 ms at  $d_s = 0.007$  to 131.4 ms at  $d_s = 0.05$ .  $\tau_{\text{syn}} = 153.3$  for drug values of  $d_s$ , only a slight increase over control (Fig. 2B). The amplitude of the IPSP does not change as  $d_s$  is varied. The rates  $d_f$  and  $r_f$  have opposing effects on the synaptic time constant; increasing  $d_f$  or decreasing  $r_f$  increases  $\tau_{\text{syn}}$  while decreases in  $d_f$  or increases in  $r_f$  will decrease  $\tau_{\text{syn}}$  (Fig. 2, C and D). As a result,  $d_f$  and  $r_f$  have no effect on  $\tau_{\text{syn}}$  when these rates are both decreased with the addition of propofol (Fig. 2D, inset). Similarly, the opposing effects on amplitude of varying  $d_f$  or  $r_f$  cannot be observed when these rates are adjusted concurrently as occurs with

propofol. Simplified expressions describing the relation between gating parameters and GABA responses are provided in an appendix in Bai et al. (1999).

Note that the complete effect of all rate changes on single IPSPs induced by either propofol or midazolam are identical (see  $k_{\text{off}} = 0.056$ , — Fig. 2A). Midazolam does not alter any of the rates of desensitization. The changes to slow desensitization with propofol have no effect on single IPSPs as seen when  $d_s$  is changed (Fig. 2B); and the effect of propofol on rates of fast desensitization negate each other, also leaving single IPSPs unaffected. In other words, changes to deactivation alone are not sufficient to explain the differences between these two drugs.

The values of  $\tau_{\text{syn}}$  that we obtain are quite large compared to the values previously described for hippocampal neurons (Buhl et al. 1995). This is partly due to the fact that the experiments of Bai et al. were performed at room temperature. Nevertheless it is important to note that these values are not absolute but depend strongly on factors such as resting membrane potential, ionic concentrations, and the history of activity at the synapse. This last condition is of particular interest here because slow receptor desensitization may have lingering effects that could effect the transmission of information at a synapse long after a period of persistent activity, particularly if transmitter levels in the synaptic cleft are elevated (Overstreet et al. 2000). The rate of recovery from slow desensitization ( $r_s$ ) is sufficiently slow that receptors beginning in this state will not return to contribute to the inhibitory response for several seconds, thus reducing the amplitude and decay time of these signals (Overstreet et al. 2000). We investigate the effect of various different initial levels of desensitization on  $\tau_{\text{syn}}$  by starting with different proportions of receptors in the closed (C) and desensitized states ( $L_2D_s$ ; Fig. 2, E and F). For these simulations, all of the parameters at the synapse are held constant at control or propofol values, and only the initial conditions of  $L_2D_s$  and C are changed. Desensitization has a significant effect on the amplitude of the IPSPs, reducing the peak of the IPSP by up to 90% (Fig. 2, E and F). In addition, desensitization also affects the synaptic time constant; under control conditions,  $\tau_{\text{syn}} = 144.3$  ms if most receptors start in the closed state and  $\tau_{\text{syn}} = 138.7$  ms if most receptors start in the desensitized state (Fig. 2E); with propofol,  $\tau_{\text{syn}} = 258.1$  ms if most receptors start in the closed state and  $\tau_{\text{syn}} = 245.1$  ms if most receptors start in the desensitized state (Fig. 2F). The effect of desensitization on  $\tau_{\text{syn}}$  becomes more complex when the synapse is activated with persistent stimulation as illustrated in the autaptic cell model (see following text). However, it is clear that with the simple gating scheme used here (developed to account for responses to rapid applications of exogenous GABA), changing the initial level of desensitization does not have a major effect on the time course of an individual IPSP.

#### Effect of persistent stimulation on synaptic response amplitude and firing frequency

Next we examine how repetitive stimulation impacts on the synaptic dynamics using an autaptic, single neuron network model. The autaptic cell model is equivalent to a larger, homogeneous, all-to-all coupled network with synchronous activity. This model can be used to determine how the frequency

of firing of cells in a network will be affected by the change in receptor kinetics induced by propofol or midazolam.

When a constant excitatory stimulus is delivered to the autaptic cell, the full effect of desensitization on network activity can be observed. For these simulations, all of the receptors begin in the ligand-unbound closed state (C) as in Fig. 2, A–D. The synaptic variables  $L_2O$  (open state),  $L_2D_f$  (fast desensitized state), and  $L_2D_s$  (slow desensitized state) are plotted in Fig. 3 to show the changes in these variables over a period of extended simulation. Slow desensitization creates a transient lasting several seconds in the synaptic variables when a tonic excitatory current drives the autaptic model. The proportion of open receptors ( $L_2O$ ) quickly decreases within a short period of time. A similar decrease in the proportion of receptors in the other states of the kinetic model is observed. The exception is the proportion of receptors in the slow desensitized state ( $L_2D_s$ ), which builds dramatically under constant stimulation due to the slow rate of return from this state.

This transient buildup of slow desensitization is also accompanied by a decrease in amplitude of the oscillating portion of the  $L_2O$  curve, which has important consequences for the network dynamics. With this stimulation the receptors never relax completely to the unbound state between firings because of the long  $\tau_{\text{syn}}$  relative to the period of firing of the autaptic cell. Thus the response can be functionally separated into two components; a low-amplitude persistent current and a time-varying current. It is this time-varying component that is affected by desensitization, becoming smaller in amplitude and duration with increasing desensitization. We measured this decrease in duration of the time-varying (phasic) response under these conditions and found that the falling phase of the open probability initially decays with a time constant of 137.1 ms but this decreases to 43.4 ms after 5 s of persistent activity as a result of the increasing desensitization (Fig. 3, inset). The time constant of decay of the phasic portion thus depends on the level of desensitization at the synapse. This is reflected by

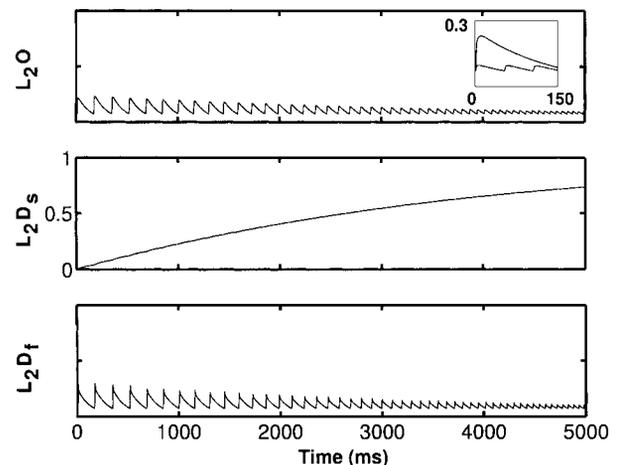


FIG. 3. Changes in the synaptic variables with persistent stimulation. The synaptic variables  $L_2O$  (top),  $L_2D_s$  (middle), and  $L_2D_f$  (bottom) are plotted for a 5 s simulation in the autapse with control values for the kinetic rates at the synapse.  $L_2O$  is the open conducting state,  $L_2D_s$  is the slow desensitized state, and  $L_2D_f$  is the fast desensitized state. To illustrate how the effective  $\tau_{\text{syn}}$  changes with activity, the inset shows smaller intervals of the  $L_2O$  curve. Inset shows the 2nd oscillation for  $L_2O$  in the train ( $\tau_{\text{syn}}$  is 137.1 ms) as compared to oscillations from the last 500 ms of the simulation ( $\tau_{\text{syn}}$  for one oscillation is 43.4 ms). For this figure, the  $\tau_{\text{syn}}$  are calculated in Clampfit (see METHODS).

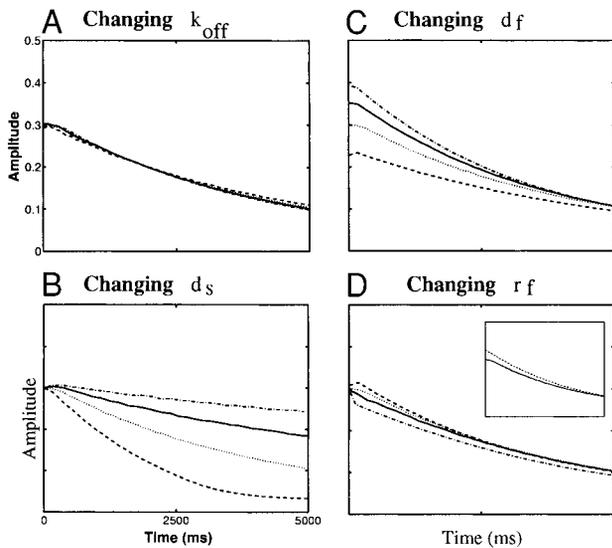


FIG. 4. Changes in amplitude of the synaptic response as individual rates are varied with persistent excitation. We plot the amplitude of the peaks in the  $L_2O$  curve over 5 s in the autaptic model with tonic excitation. Kinetic rates are varied individually, as in Fig. 2, for the rates which change upon application of propofol: A,  $k_{off}$ ; B,  $d_s$ ; C,  $d_f$ ; and D,  $r_f$  (inset,  $d_f = 1.62$ ,  $r_f = 0.12$ ), all line styles as in Fig. 2. Only  $d_s$  has a significant effect on the amplitude of the synaptic response over the interval as its value is varied. For these simulations,  $I_{app} = 1.25 \mu A/cm^2$ ,  $g_{syn} = 0.75 mS/cm^2$ . Note that  $x$  and  $y$  axes for A–D are all exactly the same as indicated.

the change in the relative  $\tau_{syn}$ , which is determined not only by the kinetics of deactivation but also by the time course of desensitization.

We proceed by varying the rates of deactivation and desensitization individually at the autapse and observe how frequency of firing and amplitude of the synaptic response respond to persistent stimulation. These simulations provide insight into how the increase in receptor desensitization with tonic excitatory input alters the effects we observe in unitary IPSPs when varying kinetic rates. As we have done for Fig. 2, in Figs. 4 and 5 each rate is varied individually to isolate its effects, and at the beginning of each simulation all receptors are initially in the closed state C.

Figure 4 shows how the amplitude of the synaptic response (as represented by the proportion of open receptors  $L_2O$ ) changes with tonic excitation (i.e., with increasing number of receptors in the desensitized state, see Fig. 3) for the rates that are affected by drug. Each point represents the peak in  $L_2O$  that occurs with each pre-synaptic action potential. Changes to  $k_{off}$  have a negligible effect on the amplitude of responses over the entire interval as is the case for individual IPSPs (Fig. 4A). This is slightly counterintuitive, as it might be expected that changes in  $k_{off}$  would alter the amplitude of responses because the change in receptor affinity alters the balance between the proportion of open and desensitized receptors. However, changes in  $k_{off}$  also affect firing frequency of the autapse (see Fig. 5), counteracting the desensitizing effects of increased affinity (i.e., decreased  $k_{off}$ ) with a decrease in the number of spikes driving the autapse to desensitize and vice versa. The effect of varying of  $d_s$  is not apparent at the onset of stimulation (as expected from its effects on single potentials), but as activity persists and desensitization at the synapse builds large disparities in amplitude at different  $d_s$  values emerge (Fig. 4B). This is due to an increase in the proportion of receptors

entering the slow desensitized state with high values of  $d_s$  resulting in a sharp decrease in the proportion of receptors left available to enter the conducting state. Changes in  $r_f$  have little effect on the peak amplitude of the inhibitory response over the interval of constant activity (Fig. 4D). Varying  $d_f$  has an initial effect on the amplitude of  $L_2O$  that gradually disappears as receptors are lost to slow desensitization, diminishing the effects seen in individual IPSPs (Fig. 4C). There is no effect on amplitude when  $d_f$  and  $r_f$  are changed concurrently as occurs with propofol, consistent with what is observed at the level of single IPSPs (Fig. 4D, inset).

Next we examine how changes in the deactivation and desensitization rates will affect the network firing period over time with increasing desensitization. In Fig. 5, we plot the duration of inter-spike intervals for consecutive action potentials produced with tonic excitation. In all cases, network period diminished with time as a consequence of receptors gradually being sequestered in the slow desensitized state. Changes in the ligand unbinding rate  $k_{off}$  significantly affect the period of firing in agreement with its effect on IPSPs, although this effect decreases over the interval as the level of desensitization at the synapse increases (Fig. 5A). In contrast the rate constants governing the fast desensitized state ( $d_f$ ,  $r_f$ ), which also had an effect on  $\tau_{syn}$  in single responses, have very little effect on firing frequency (Fig. 5, C and D). Changes to  $d_s$  have a large effect on firing frequency even though they do not significantly affect the duration or amplitude of single IPSPs. This is because  $d_s$  alters the amplitude of synaptic response during repetitive firing because the reduction in the proportion of open receptors with high  $d_s$  values reduces inhibition at the synapse, affecting the firing frequency of the autapse (Fig. 5B).

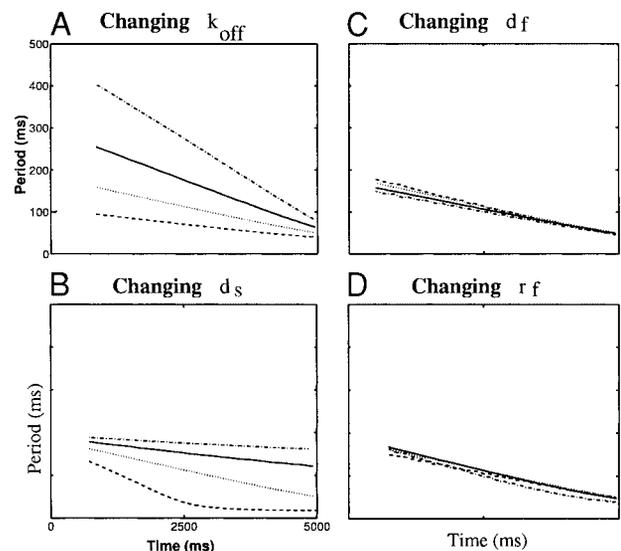


FIG. 5. Changes in firing period of the autapse with persistent excitation as individual rates are varied. We plot consecutive inter-spike intervals for the autapse for 5 s in the autaptic model with constant excitation. Kinetic rates are varied as in Fig. 4: A,  $k_{off}$ ; B,  $d_s$ ; C,  $d_f$ ; and D,  $r_f$ . Line styles according to parameter values as in Fig. 2. Both  $k_{off}$  and  $d_s$  have significant effects on firing frequency over the interval when they are varied. Again for these simulations,  $I_{app} = 1.25 \mu A/cm^2$ ,  $g_{syn} = 0.75 mS/cm^2$ . Note that  $x$  and  $y$  axes for A–D are all exactly the same as indicated.

### Drug decreases network frequency

Next we consider the combined effect of all of the kinetic rate changes induced by propofol and midazolam on firing frequency in the autapse. Table 3 shows a snapshot of the network period obtained using the parameter values given in Table 1. Three different initial levels of desensitization (i.e.,  $L_2D_s$ ) are used and network period is taken as the second inter-spike interval obtained. (The 1st inter-spike interval would not yet reflect the effect of desensitization because it would use the first spike in which the synapses are still “naive” with respect to desensitization). The addition of propofol reduces the firing frequency of the autapse over all levels of desensitization. This is expected because the network frequency is determined by the duration of the synaptic time constant, and propofol increases  $\tau_{syn}$  by reducing the ligand-unbinding rate,  $k_{off}$ . Because midazolam also increases  $\tau_{syn}$  by reducing  $k_{off}$ , the effect of midazolam would also be to reduce the firing frequency. However, because the level of desensitization affects the frequency (see Figs. 3 and 5), the numbers would not be exactly the same.

### Alteration in the conditions necessary for coherent activity by drug

Work by Chow and colleagues (Chow et al. 1998; White et al. 1998) shows that the autaptic model is useful for predicting the ability of neurons in heterogeneous multi-neuron networks to synchronize. Specifically, White et al. show that the  $\tau_{syn}$  versus T relationship can be used to classify networks into two regimes, which they term tonic and phasic, that have different synchronization properties. In the tonic regime, the synaptic strength has only a weak time-varying component and the network period T is independent of  $\tau_{syn}$  (i.e., a relatively constant  $\tau_{syn}$  vs. T curve). In this regime, the IPSPs lose their ability to entrain the network into a coherent ensemble. When the synaptic responses in the network vary strongly with time, the network is classified as being in the phasic regime. Here network period will depend sensitivity on  $\tau_{syn}$  (i.e., a nonconstant, curved  $\tau_{syn}$  vs. T relationship), and neurons in the network can be synchronized.

Modelling  $\tau_{syn}$  changes as changes in  $k_{off}$  (see Eq. 1 in Bai et al. 1999), we have shown previously that propofol alters the responsiveness of the autapse to changes in the duration of inhibitory synaptic input (Baker et al. 2001). In other words, we found that propofol makes the period, T, sensitive to  $\tau_{syn}$  (i.e., phasic regime) and hence predicts synchrony in larger, heterogeneous networks using White et al.’s theoretical insights (White et al. 1998). Furthermore, this sensitivity is preserved with varying levels of desensitization (not shown). This change in the responsiveness of the autapse is explained

TABLE 3. Network period changes with varying levels of desensitization in the autapse

Initial $L_2D_s$	Firing Period, ms	
	Control	With propofol
0.1	162.8	279.4
0.5	104.0	181.0
0.9	18.6	19.8

by considering how the inhibitory response changes with increasing desensitization, as shown in Fig. 3. With repetitive stimulation, the synapse becomes strongly desensitized and the time-varying component of IPSPs becomes small (Fig. 3, *inset*). In this highly desensitized state, the level of the persistent synaptic inhibition continues to alter the firing frequencies of cells in the network, but the duration of the IPSPs will not determine the network frequency. This is because the time-varying component of the inhibition will be too small to significantly affect firing rate. However, at lower levels of desensitization, as occurs at the beginning of onset of excitation (Fig. 3, *inset*), IPSPs will have a strong time-varying component that will entrain the network period, and under these conditions the frequency of firing of the model neurons will change depending on the time constants at synapses in the network. Because propofol decreases the entry rate into the desensitized state, the synapses will desensitize more slowly, thus preserving the phasic response or the strong time-varying component.

### Propofol and midazolam have qualitatively different effects on synchrony and frequency in inhibitory networks

Let us now consider heterogeneous, two-cell networks and explain our observations on synchrony and frequency using the insight gained in the preceding text. In previous theoretical work, White et al. (1998) show that networks with heterogeneity in their excitatory drive, such as the two-cell model we consider, differ in their ability to synchronize depending on whether the model parameters produce network outputs that are in the phasic or tonic regime. When the synapse has a small time-varying component (tonic regime), the cells will fire asynchronously because the synaptic currents influence firing frequency but do not entrain the model neurons. When the time-varying component of the synaptic current is large (phasic regime), the network can display different types of behavior: when inhibition is very strong, some cells in network do not fire at all (suppression); when inhibition is slightly weaker, cells fire on some cycles of the network frequency (harmonic locking); and with the correct balance of inhibition and excitation, synchrony is obtained (all cells in network are phase-locked). We can characterize the state of the two-cell network by plotting the coherence measure or correlation (see METHODS) over a range of values of the maximal synaptic conductance ( $g_{syn}$ ) and excitatory drive ( $I_{app}$ ). In general, at any given  $g_{syn}$ , the correlation is zero when one of the cells is not firing (suppression); as  $I_{app}$  increases, correlation approaches one (synchrony); and as  $I_{app}$  is increased further, the correlation declines (asynchronous behavior).

Fig. 6 shows correlation maps for two-cell networks with heterogeneity in  $I_{app}$  with control synapses or synapses with propofol or midazolam. The maps are colored to indicate the frequency of the faster firing cell; however both cells fire at roughly the same frequency because the heterogeneity in input drive between the two is weak (see METHODS). The simulations were run until desensitization reached steady state (40 s), i.e., equilibrium desensitization, and then the correlation was measured over the final second of the simulation (see METHODS). The control network (Fig. 6A) has a region of high correlation corresponding to synchronous activity over a range of  $I_{app}$  values lower than those that produce synchronous activity in

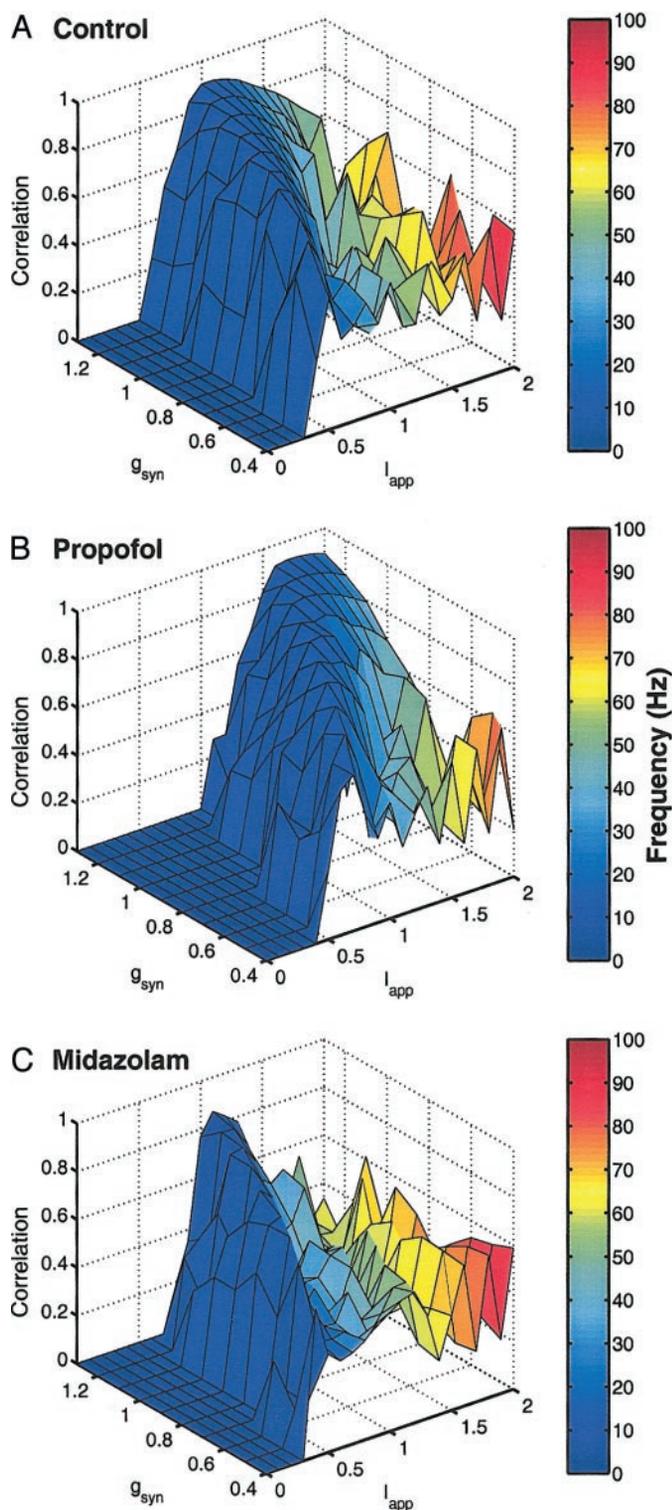


FIG. 6. Correlation mapped over a range of  $g_{syn}$  and  $I_{app}$  in a 2-cell network with heterogeneity in input excitation. The coherence measure or correlation (see METHODS) is mapped for synapses under control conditions (A), with propofol (B), and with midazolam (C). For each set of parameters, the simulation is run for 40 s to allow synapses in the network to become fully desensitized, and then the correlation is determined over a 1 s interval. The map is coloured to indicate frequency of firing of the faster firing cell. The correlation maps give a qualitative picture of the state of the network as the parameters are varied. When correlation equals 0, there is suppression in the network; as excitation ( $I_{app}$ ) increases, correlation reaches a peak and the network becomes phase-locked; and as excitation is increased further, the cells in the network fire asynchronously (the jagged region of the correlation map).

the network with propofol (Fig. 6B). This is a result of the reduction in desensitization with the addition of propofol. The increase in synaptic inhibition with propofol requires an increase in the minimum amount of excitatory drive ( $I_{app}$ ) to elicit (synchronous) firing for a given  $g_{syn}$ . With propofol, the network exhibits more phasic behavior than the control network (because it is not as desensitized), and it has a larger range of suppressive and synchronous activity over  $g_{syn}$  and  $I_{app}$ , while the control network has a larger region of asynchronous activity, corresponding to tonic behavior. However also note that with propofol the network synchronizes at significantly higher  $I_{app}$  values than under control conditions.

From the autaptic cell model (Fig. 5, Table 2) (Baker et al. 2001), we would expect that propofol would cause the network to fire at lower frequencies than control for the same values of  $g_{syn}$  and  $I_{app}$ . This occurs because propofol lowers  $k_{off}$ ; this lengthens the synaptic time constant. In addition, propofol lowers  $d_s$ , reducing receptor desensitization. This pushes the network into the phasic regime where network frequency is more closely tied to  $\tau_{syn}$ , so that the decrease in  $k_{off}$  with propofol has an even greater effect on frequency. This is confirmed in our two-cell model simulations (Fig. 6, A and B). If a high level of synchrony is considered to be a correlation value greater than 0.7, then the control network is synchronous at frequencies in the 8–45-Hz range over the range of parameter values examined, while the network with propofol has high correlation over frequencies at approximately 6–37 Hz (i.e., smaller range) over the range of parameter values plotted. We examine correlation in networks with varying initial levels of desensitization (not shown) as done in Table 3. The trend observed for the equilibrium desensitization correlation map (Fig. 6) is preserved; the network with propofol requires a higher level of excitatory drive to fire at all values of  $g_{syn}$  and levels of desensitization, and it is possible for correlation to be maintained for larger  $I_{app}$  values. In summary, the requirement for larger  $I_{app}$  to elicit synchronous firing is due to the excitatory input having to overcome the larger inhibition (produced with propofol) but correlation is possible and can occur at these larger  $I_{app}$  values because of the phasic effect of the decreased desensitization with propofol. In contrast, this is not the case with midazolam.

Changes in receptor kinetics with midazolam also alter network state but with different effects on network dynamics as compared with propofol. Midazolam would also influence firing frequency of the network relative to control because of the increased  $\tau_{syn}$  associated with the reduced  $k_{off}$  value. However, midazolam does have a subtle effect on macroscopic desensitization despite its lack of effect on the desensitization rates. Increasing the affinity of the receptor (by decreasing  $k_{off}$ ) without a concurrent decrease in the rate of entry into slow desensitized state ( $d_s$ ), actually promotes entry of receptors into the desensitized state over the level observed for control synapses by trapping ligand on the receptor, allowing more opportunities for the receptor to enter the slow desensitized state. Thus we would expect that the phasic component is lost more quickly so that less correlation would be possible, and we would expect a greater range of asynchronous behavior in the correlation map with midazolam as compared to control.

Results for the two-cell network with midazolam are shown in Fig. 6C, and confirm our predictions described in the preceding text. Midazolam produces a larger region of asynchro-

nous activity (which is associated with the tonic regime) than the control network (Fig. 6A). In addition, networks with midazolam synchronize over a much smaller range of the parameter space than control. Thus midazolam has a qualitatively different effect on network behavior compared to propofol despite the fact that they have similar effects on single IPSPs (Fig. 2A).

Overall these results provide insight into which rate changes predominate in determining behavior of networks. At low levels of desensitization, rate changes that affect affinity (such as the ligand unbinding rate,  $k_{\text{off}}$ ) determine network behavior because in the phasic regime, factors that determine  $\tau_{\text{syn}}$  have their greatest effect. For higher levels of desensitization, the rates controlling the level of slow desensitization (i.e.,  $d_s$ ) govern network behavior because it is the amplitude of the steady-state inhibition in the tonic regime that paces the network.

### Complex interactions and theoretical insights

The different network responses in Fig. 6 can be further explained. Note that with midazolam, the network actually requires a slightly higher level of excitatory drive to elicit activity than control (Fig. 6, A vs. C). This is not an intuitive observation as networks with midazolam have an increase in desensitization over control (as explained in the preceding text), and therefore might be expected to require less excitation to elicit activity than control networks. However, this phenomenon is explained in our model, and it highlights the complex dynamic effects of changes in slow desensitization and receptor affinity on network activity. We return to the autaptic cell model and compare the amplitude and shape of the open probability ( $L_2O$ ) curve for the three different autapses at equilibrium desensitization (Fig. 7). With midazolam, the slight increase in desensitization does not result in an overall decrease in open probability (i.e., the curve does not shift down the y axis), but a specific decrease in the amplitude of the time-varying (phasic) component of the inhibitory response

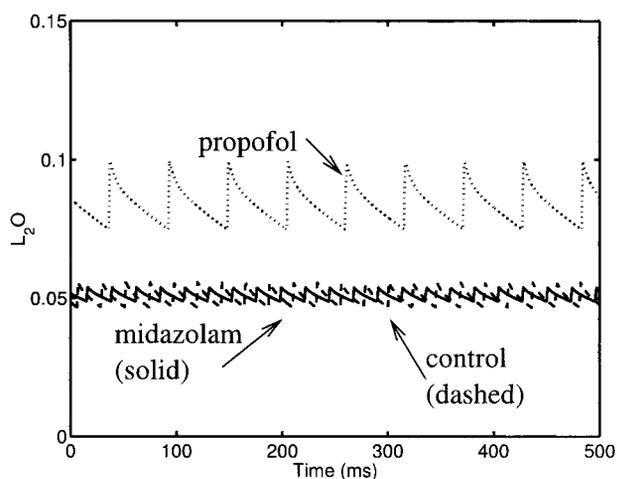


FIG. 7. Network behavior is determined by changes in the synaptic variable  $L_2O$ .  $L_2O$  is plotted over 500 ms at the autapse for control parameters (---), with propofol (· · ·) and with midazolam (—) at equilibrium desensitization (40 s) to illustrate how changes in the kinetic rates, which shape the  $L_2O$  curve, determine the behavior manifested in the different 2-cell networks. For these simulations,  $I_{\text{app}} = 1.25 \mu\text{A}/\text{cm}^2$ ,  $g_{\text{syn}} = 0.75 \text{mS}/\text{cm}^2$ .

compared to control levels (Fig. 7). This is apparent because the mean value of  $L_2O$  over time is similar for midazolam and control (0.0505 for control vs. 0.0511 for midazolam). The change in the amplitude of the phasic portion of the  $L_2O$  curve is due to increased desensitization, reducing the peaks in the  $L_2O$  curve, and the increased  $\tau_{\text{syn}}$ , which prevents  $L_2O$  from relaxing back to control levels between stimuli. These changes in the synaptic dynamics, that are tied to the kinetic rates  $k_{\text{off}}$  and  $d_s$ , are responsible for degrading synchrony in the network (Fig. 6, A and C). In addition, the reduction in the phasic response with midazolam increases overall inhibition along with desensitization, since the value of  $L_2O$  at the minima of the curve is actually higher with midazolam versus control (Fig. 7). It is this increase in the tonic level of inhibition that necessitates more excitation to elicit firing in the network with midazolam. These changes exemplify the phasic to tonic switch in behavior that occurs with addition of midazolam. In contrast, with propofol both the amplitude and mean level of the inhibitory response are increased dramatically over control because of the effects of slowing the rates of slow and fast desensitization in addition to lengthening  $\tau_{\text{syn}}$  (Fig. 7). The differences between the propofol, midazolam and control networks illustrate the complex dynamic effects of changes in slow desensitization and receptor affinity on network activity. In summary, the interplay between desensitization and deactivation kinetics at the synapse and their resultant effects on the behavior of neurons in networks is a complex, nonlinear relationship that requires a theoretical approach such as the one we have presented in this study.

## DISCUSSION

### Summary of model results and predictions

Ligand-gated receptor kinetics determine the duration and amplitude of synaptic currents. They are also important for shaping the activity patterns of neuronal networks, particularly when the kinetics of the synaptic receptors are slow. In this paper, we show how changes in the kinetics of slow desensitization and deactivation of the  $\text{GABA}_A$  receptor in the absence or presence of the sedative-amnestic midazolam and the hypnotic propofol impact on the frequency and synchronized oscillatory activity in a model of a hippocampal inhibitory network. Our model predicts that propofol dramatically decreases the frequency of network firing and increases the amount of excitatory drive necessary for synchronous activity, resulting in a wider range of synaptic strength values for which synchrony is observed at larger  $I_{\text{app}}$  (Fig. 6, A and B). In contrast, networks with midazolam synchronize at levels of excitation similar to those required for synchrony in the control network, although at lower frequencies than for control. Networks with midazolam also synchronize over a smaller range of parameters than control networks (Fig. 6, A and C). These results show that  $\text{GABA}_A$  receptor desensitization could play a key role in mediating the different behavioral effects of these drugs by altering the ability of inhibitory networks to synchronize.

### Brain slice studies, EEG studies, and model predictions

Our work represents a critical first step in linking alterations in channel kinetics with behavioral changes. There are well-

documented effects of anesthetic agents on intraoperative electroencephalography (EEG) and evoked potentials (EP) (e.g., see Sloan 1998). EEG studies have shown a marked decrease in power of the high frequency bands (i.e., gamma) and a corresponding increase in lower-frequency bands with propofol anesthesia (Fiset et al. 1999). Propofol anesthesia in human subjects can be reversed by physostigmine, an anticholinesterase inhibitor that could potentiate excitatory modulation of inhibitory networks in the hippocampus mediated by cholinergic pathways (Meuret et al. 2000). Thus if we make a huge speculative leap from the results of our simulations, we can say that in some sense our model predicts this because it shows a significant increase in correlated activity (putatively corresponding to a responsive state) with propofol compared to control at higher  $I_{app}$  values (i.e., potentiated excitation with physostigmine). Most interestingly, Ma et al. (2002) have shown that the cholinergic septohippocampal system can influence general anesthesia because potency of a general anesthetic was increased when the system was suppressed. Rhythmic 30–50 Hz states and anesthetic behavioral states were correlated. Clearly, the brain is a high-dimensional dynamical system whose behavior requires careful analyses to adequately understand because all levels in the hierarchical organization of CNS contribute to the EEG output. Network models such as ours start to provide a link in translating between the levels of organization in the CNS.

Whittington et al. (1995) showed that purely inhibitory networks in hippocampal brain slices could support the production of synchronized gamma oscillations. Experiments investigating the effect of propofol and midazolam on such oscillations have been done (Faulkner et al. 1998; Whittington et al. 1996). They observed a decrease in frequency with propofol and midazolam, but a decrease in coherence only with propofol. Their results can be compared with our simulations. As the parameters in their experiments were such that they obtained coherent activity under control conditions, then at the same parameter set (i.e. same value of  $g_{syn}$  and  $I_{app}$ ) for preparations with propofol, our model predicts a decrease in correlation and frequency of activity and similar levels of correlation with midazolam. Because network coherence is possible over a range of conditions in vitro, the value of the parameters relevant to coherence (i.e., excitation, inhibitory coupling strength, level of desensitization at network synapses) must be carefully considered. The advantage of our approach is that an understanding of the complexity of the nonlinear, interacting dynamics has been obtained using previous theoretical insights of inhibitory network dynamics. Therefore we can exploit the understanding of anesthetic action obtained from our model to predict how manipulating the level of desensitization in the slice should affect network synchrony both with and without drug. For example, pharmacological agents that increase desensitization of synapses [such as GABA uptake inhibitors (Overstreet et al. 2000)] should reduce the responsiveness to changes in  $\tau_{syn}$  (such as that induced by midazolam) in an autaptic cell culture. Our results also predict that as excitation in hippocampal slice is enhanced (e.g., with cholinergic agonists) synchronized oscillations should be maintained for higher levels of excitation with propofol than under control conditions.

### *Synaptic plasticity and the shaping of inhibitory network activity*

Synaptic depression is observed in various cortical regions including hippocampus (Galarreta and Hestrin 1998; Maccaferri et al. 2000), and dynamic synapses have important roles to play in neural computation (Abbott et al. 1997; Tsodyks and Markram 1997). However, post-synaptic receptor desensitization is one of many mechanisms by which the phenomenon of synaptic depression can be expressed. There are various forms of short-term plasticity that contribute to activity-dependent depression of inhibitory postsynaptic currents. Evoked IPSCs between pairs of cultured rat hippocampal neurons showed robust paired-pulse depression (PPD) using interpulse intervals from 25 to 2,000 ms (Jensen et al. 1999). PPD was influenced by a decrease in release probability due to reduced presynaptic  $Ca^{2+}$  influx, presynaptic GABA<sub>B</sub> receptors, potassium channel function, in addition to post-synaptic receptor function. Recordings in CA1 hippocampal pyramidal cells indicated that shifts in the chloride concentration gradient also contribute to activity-dependent depression (McCarren and Alger 1985). In addition, slow GABA<sub>A</sub> receptor desensitization (Behrends et al. 2002; Overstreet et al. 2000) contributes to PPD depression. Recently, PPD has been observed between basket and granule cells in the dentate granule of rat hippocampus with a presynaptic mechanism (Bartos et al. 2001; Hefft et al. 2002), although a slow recovery time course was also apparent (Bartos et al. 2001). Interestingly, Hefft et al. (2002) concluded that different types of presynaptic modulation were possible because they found that their depression was independent of release probability.

We have used the kinetic model of Bai et al. (1999) as this model specifically addressed differences between GABAergic currents in cultures with and without propofol. This detailed description of the synapse allows us to address network mechanisms of anesthesia, as well as isolating the effect of slow desensitization on network activity by comparing the actions of propofol and midazolam. However, the model proposed by Bai et al. has a larger time constant of synaptic decay than is normally considered in most models of inhibitory networks [i.e.,  $\tau_{syn} = 145$  ms vs. 10–40 ms in White et al. (1998)]. Such slowly decaying inhibitory responses are found in hippocampal interneurons (Banks et al. 1998; Ouardouz and Lacaille 1997) although it is unclear what role these currents may have in the generation of hippocampal rhythms. As a result of this longer time constant, we observe a large range of coherent activity in the theta frequency range (8–15 Hz), consistent with previous modelling work which has proposed that this slower current may play a role in the generation of theta rhythm in hippocampus (Banks et al. 2000; White et al. 2000). There is strong evidence in cultures for desensitization of GABAergic synapses even at very low concentrations of GABA in the extracellular space ( $\approx 1 \mu M$ ) (Overstreet et al. 2000); this suggests that inhibitory synapses could be considerably desensitized even during quiescence in vivo. Indeed, the level of desensitization of synapses plays a significant role in determining network frequency (see Table 3). Furthermore, both synaptic and extrasynaptic receptors are activated by GABA. These receptors have different kinetic and pharmacological profiles (Bai et al. 2001; Banks and Pearce 2000) and could influence our network simulations. However, the effects of propofol on

the evoked current were qualitatively similar to the effects on synaptic currents. The kinetic parameters used for our models were derived from the best available data that qualitatively reflect the effects of propofol and midazolam on synaptic receptors. In summary, given the important role that desensitization can play in determining the conditions necessary for synchronized oscillations in inhibitory networks (as predicted by our network models), characterization of the time course and extent of desensitization in functional *in vitro* networks is desirable.

#### *Changes in receptor kinetics describe how drugs affect the functional properties of GABA<sub>A</sub> receptors*

We show that by using a precise definition of kinetic properties such as time course of deactivation and desensitization of GABA<sub>A</sub> receptors and describing how these kinetics are affected by drug action, a mechanistic understanding of how a given drug affects system activity, and thus behavior, can be gained. However, the kinetics of receptors are dependent on structural properties such as subunit composition, meaning the highly selective expression of particular receptor subunits on a given neuron tailors each synapse within a circuit for a particular dynamic role in controlling network output and thus behavior. Molecular biological identification of the specific isoforms of the GABA<sub>A</sub> receptor present in various neuronal circuits is underway (Macdonald and Olsen 1994; Mehta and Ticku 1999; Vicini 1999), and this work has led to mutation studies that have given insight into how drug interactions with receptors at the molecular level can affect behavior (Loew et al. 2000; McKernan et al. 2000). While these studies have identified the specific circuits responsible for disparate effects of the anesthetic, they are not able to explain the mechanism for the observed changes with addition of drug. Such information is required for improving drug design, dosage, and delivery protocols. We propose that an understanding of how the kinetic properties of any given isoform of the GABA<sub>A</sub> receptor contributes to network function can be exploited to design drugs that selectively modify a desired behavior, while leaving other functions intact.

Receptor subunit mutation studies also provide an opportunity to exploit the dynamic effect of anesthetics on post-synaptic currents to test models of activity generation and maintenance in neuronal networks. For example, recent studies of sleep spindles in thalamus used differences in synapse-specific GABA<sub>A</sub> receptor subunit expression and differential modulation of mutated and wild-type receptor subunits by benzodiazepines to test theoretical models of spindle propagation in slice (Sohal et al. 2001). In systems where the synaptic dynamics are thought to subservise some emergent network property, characterization of the effect of anesthetics at the specific receptor isoforms present at those synapses could be used to verify or refine models that describe the generation of the emergent network behavior.

#### *Mathematical models as a tool for understanding mechanisms of anesthesia*

We have shown that mathematical modelling can be a valuable tool for furthering our understanding of how anesthetic drugs might mediate their effects. Although the networks we

study are small, they allow us to investigate fully how the network dynamics are shaped by the kinetics at the synapse. As network size increases, the number of parameters required to describe the system grows dramatically, making detailed analysis such as we have carried out here difficult. However, preliminary data from additional simulations that we have performed suggest that our findings hold for 10-cell networks as well (Baker and Skinner 2002). In their simulations, White et al. (1998) find that 2-, 10-, and 100-cell networks with weak heterogeneity and all-to-all coupling behave similarly, and we expect the same to hold true for our model. Future work will include studying the effect of increased heterogeneity in input excitation and heterogeneity in other parameters as well as considering larger networks of appropriate architectures.

Previous attempts to explain neural mechanisms of anesthesia have focussed on drug interactions at the molecular and cellular level. Most anesthetics have been shown to interact with a wide range of molecular targets (Franks and Lieb 1998), making it difficult to determine how these various changes cooperate at the level of neuronal networks to produce the behavioral effects of these drugs. By taking advantage of previous modelling work, we have developed a mechanistic understanding of how modulation of GABA<sub>A</sub> receptor desensitization by propofol and midazolam can affect the generation and maintenance of synchronous oscillatory activity in inhibitory networks. Our results suggest that differences in dynamic modulation of this circuitry may contribute to the differences in the macroscopic effects of these drugs.

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