Mechanism of psychoactive drug action in the brain: Simulation modeling of GABA\textsubscript{A} receptor interactions at non-equilibrium conditions.

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Abstract.

Synaptic transmission requires that the binding of the transmitter to the receptor occurs under rapidly changing transmitter levels, and this binding interaction is unlikely to be at equilibrium. We have sought to numerically solve for binding kinetics using ordinary differential equations and simultaneous difference equations for use in stochastic conditions. The reaction scheme of GABA interacting with the ligand-gated ion-channel demonstrates numerical stiffness. Implicit methods (Backward Euler, ode23s) performed orders of magnitude better than explicit methods (Forward Euler, ode23, RK4, ode45) in terms of step size required for stability, number of steps and cpu time. Interestingly, upon solving the system of 8 ordinary differential equations for the GABA reaction scheme we observed the existence of low dimensional invariant manifolds that may have important consequences for information processing in synapses. We also describe a mathematical approach that models complex receptor interactions in which the timing and amplitude of transmitter release are noisy. Exact solutions for simple bimolecular interactions that include stoichiometric interactions and receptor transitions can be used to model complex reaction schemes. We used the difference method to investigate the information processing capabilities of GABA receptors and to predict how pharmacological agents may modify these properties. Initial simulations using a model for heterosynaptic regulation shows that signal to noise ratios can be decreased in the presence of background presynaptic activity both in the presence and absence of chlorpromazine. These types of simulations provide a platform for
investigating the effect of psycho-active drugs on complex responses of transmitter-receptor interactions in noisy cellular environments such as the synapse. Understanding this process of transmitter–receptor interactions may be useful in the development of more specific and highly targeted modes of action.
1. Introduction.

Although much is known about the control of transmitter release, the binding of the transmitter to the postsynaptic receptor is still poorly understood in central synapses; these are specialized nerve structures that transfer information through the secretion of chemical transmitters. Synaptic signaling events are capable of performing high order computations; they respond selectively to patterned input and sustain changes in response to stimuli over multiple time scales. However, the small volume of the synapse makes the analysis of signaling complex because synaptic processes are strongly affected by stochasticity. Whereas properties such as bistability and pattern selectivity persist as sharp thresholds in the deterministic situation, but the transition to upper and lower is broadened and patterns get degraded when stochasticity is considered. Additionally, in some regimes processes such as stochastic resonance enhances detection of firing patterns.

The inaccessibility and microscopic scale of synapses makes it difficult to study the kinetics of signal reception at high resolution. For example, there is still considerable controversy about whether the amount of transmitter released in a single quantum is sufficient to saturate the postsynaptic sites and the nature of the variation in transmitter release. Furthermore, neurotransmitter transients may contribute differentially in large and small synapses to the variability of the inhibitory post-synaptic potential decay.
Using both a modeling and an experimental approach the authors showed that the variability of GABA transients conducted by GABA_A receptors using chloride ions to generate inhibitory currents, in small synapses is needed to account for the variability seen in inhibitory post-synaptic potential (IPSP) decay, whilst in large synapses the stochastic behavior of 36 pS channel results in the variability of IPSP decay. Because of the importance of this initial step in signal transduction, there have been many elegant and technically challenging attempts to characterize transmitter-receptor interactions in vivo. These have yielded several results for the role of receptor desensitization in shaping postsynaptic currents that could not have been predicted from conventional binding studies performed at equilibrium or simple kinetic schemes. From these and other molecular studies it is clear that allosteric and enzymatic regulation can lead to complex binding characteristics that may be important at many levels of integration up to cognitive learning. Neuronal receptors are a major target for psychoactive drugs, therefore understanding modes of drug action in the central nervous system will require better description of binding and electrical conduction mechanisms in stochastic driven environments. Understanding this process of transmitter–receptor interactions may aid in the development of more specific and highly targeted modes of action.

2. Modeling transmitter binding to GABA-gated ion channels.

Both ligand binding studies and single channel analysis have been used extensively
to describe neurotransmitter binding to ligand gated ion channels. These studies provide
detailed kinetic information relating receptor transitions and binding of transmitters to
different receptor states. Monte Carlo simulations, used to model transmitter timecourse
in the cleft and subsequent receptor activation, show the important relationships between
the peak amplitude of the postsynaptic response, stochastic variability of the response,
diffusion and clearance of transmitter, agonist affinity and its stoichiometry of interaction

One of the most extensive networks of receptors in the brain for inhibitory
transmission are a set of GABAergic cells that are crucial in controlling the activity of
neuronal networks. Communication occurs through release of gamma-aminobutyric acid
(GABA), which is an inhibitory neurotransmitter. The subcellular localization and
intrinsic properties of heteropentameric GABA_A receptors constitute major sources of
diversity in GABA-mediated signaling. The predominant mechanism of signal
transmission at the synapse is via chemical exchange from the presynaptic cell to the
postsynaptic cell. The narrow gap between these is called the synaptic cleft, into which
neurotransmitters, such as GABA, are released. Upon release, the neurotransmitter is
rapidly removed from the synaptic cleft. In order to understand the chemical signal, the
postsynaptic cell must be equipped with transmitter receptors. We chose to study the
ligand-gated GABA_A receptor because it has been extensively studied using single
channel analysis of evoked currents. These kinetic studies provide detailed kinetic
schemes that describe transitions to and from multiple receptor states. Furthermore, the GABA$_A$ receptor is thought to play an important role in many cognitive functions of the brain, and it has been a major target site for the development of anti-depressant and anti-convulsant drugs. This has led to a good understanding of the effects of these drugs on the proposed kinetic scheme of receptor activation. However, many of these characterizations have been carried out in reduced preparations in which the agonist is delivered using single or paired pulses. The natural release of transmitters, including GABA, is more commonly a series of pulses with a noisy distribution.

Another interesting aspect of GABA neurotransmission is that sensory stimulation can lead to the generation of gamma oscillations in the massed cortical that require GABA$_A$ receptor activation and can occur even when glutamergic transmission is blocked. Certain stimulation paradigms (e.g., tetanic stimulation at twice the threshold for gamma) result in a transition to beta frequencies (10-30 Hz). Studies using GABA$_A$ receptor beta3 subunit deficient mice (beta3/-) showed an important role of GABAergic inhibition in the generation of network oscillations in the olfactory bulb (OB). These studies revealed that the disruption of GABA$_A$ receptor-mediated synaptic inhibition of GABAergic interneurons and the augmentation of IPSCs in principal cells resulted in increased amplitude theta and gamma frequencies. This rhythmic spiking in cortical networks occurs through excitatory and inhibitory cell interactions generated by low driving of inhibitory cells. Simulations that investigated the role of noise in these
networks showed that noisy spiking in the excitatory-cells adds excitatory drive to the inhibitory cells that may lead to phase walkthrough. Noisy spiking in the inhibitory-cells adds inhibition to the excitatory-cells resulting in suppression of the excitatory-cells. Because of this important property of noise, one goal of the current study is to help understand receptor-tuning properties during different stimulation frequencies and in which the peak to peak transmitter release amplitudes are also changing. These tools will also allow us to measure the frequency response properties of the receptor and determine if a receptor in a noisy environment will preferentially resonate to the beta or gamma frequencies.

We describe here the use of two numerical methods to solve for non-linear equations governing transmitter binding and receptor transition states: Stiff Ordinary Differential Equations that describe the reaction scheme of GABA interacting with the ligand-gated ion-channel and Analytic formalisms to use in difference equations to solve for transmitter-receptor interactions in noisy environments.

3. Ordinary Differential Equations.

Our numerical investigation revealed that the model exhibits stiffness; a characterization of stiffness will be provided. In this particular situation, this phenomenon means that the dynamical system reduces from being eight-dimensional
(corresponding to eight chemical concentrations) to seven, and then to five. This dimension reduction may have biological implications, which we conjecture upon and would like to investigate further.

Stiff dynamical systems are exceedingly difficult to integrate using standard explicit methods, and require very small time-steps in order to maintain numerical stability. The use of adaptive algorithms does not significantly alleviate this problem; the integrator selects an unreasonably small allowable step-size. Heuristically, the time-step is selected to resolve the behavior of the fastest transient in the system. However, this transient may not be the dominant mode and may contribute negligibly to the overall dynamics of the system. The net result is a significant waste of computational effort. A well-known strategy to numerically integrate stiff systems is to use an implicit method. We first provide a working definition of stiffness, and then demonstrate through simple examples the difference between using explicit and implicit methods.

We began our investigation by using standard (explicit) algorithms to numerically simulate the model. During the experiments we encountered stability problems, which necessitated the use of stiff solvers. The success of these latter algorithms led us to characterize the model as stiff. Once we had stable and accurate algorithms to study the model, we observed the existence of invariant manifolds.

The organization of this paper is as follows. We begin with a brief description of
the model in question. We describe stiffness and present examples, which demonstrate the power of implicit methods. We present the results of our investigations of the GABA model. We end this paper with a tentative biological explanation for the observed phenomena, as well as suggested experiments to verify our predictions.

3.1. The GABA reaction scheme

The GABA reaction scheme considered here models the various concentrations of GABA, bound and unbound receptor, and desensitized and open states after the simulated release of a GABA pulse.

The GABA reaction scheme is based on the classical bimolecular reaction of Michaelis-Menten, suitably adapted to account for reversible reactions and multiple receptor states. The Michaelis-Menten reaction describes a substrate R, reacting with an enzyme N, which form a complex RN, soon converted into a product P and the enzyme.

Where \( k_1, k_1, \) and \( k_2 \) are the rate constants of the reaction. The Law of Mass Action says that the reaction rate is proportional to the product of the concentration of the reactants (we use the same letters to denote both the reactants and their respective concentrations, with the exception [RN ]= C). This leads to the Michaelis-Menten equations:

\[
\begin{align*}
\frac{dR}{dt} &= -k_1 RN + k_2 C, \\
\frac{dE}{dt} &= -k_1 ES + (k_1 + k_2) C, \\
\frac{dC}{dt} &= k_1 ES - (k_1 + k_2) C, \\
\frac{dP}{dt} &= k_2 C.
\end{align*}
\]
The GABA binding and state transition scheme.

**Binding Reactions**

- \( R + N \xleftrightarrow{2\cdot kon / koff} B_1 \)
- \( B_1 + N \xleftrightarrow{kon / 2\cdot koff} B_2 \)
- \( D_s + N \xleftrightarrow{q / p} D_f \)

**State transitions**

- \( B_1 \xleftrightarrow{b_1 / a_1} O_1 \)
- \( B_1 \xleftrightarrow{d_1 / r_1} D_s \)
- \( B_2 \xleftrightarrow{b_2 / a_2} O_2 \)
- \( B_2 \xleftrightarrow{d_2 / r_2} D_f \)

The GABA scheme assumes all the reactions are reversible (i.e. all arrows in the reactions are double pointed). One of the modifications of Michaelis-Menten in the GABA scheme stems from the fact that the end product \( P \) is a bound receptor. In this way, \( \text{GABA} \equiv N \) is not a catalyst; it is used up in the reaction. So, the second half of the reaction is not present. A further adaptation is that the GABA scheme involves processes in which the receptor \( R \) has multiple GABA binding sites. The GABA reaction variables can be found in Table 2.

### Table 2: GABA scheme variables

<table>
<thead>
<tr>
<th>( R )</th>
<th>Receptor concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )</td>
<td>Ligand (GABA) concentration</td>
</tr>
<tr>
<td>( B_1 )</td>
<td>First bound state concentration</td>
</tr>
<tr>
<td>( B_2 )</td>
<td>Second bound state concentration</td>
</tr>
<tr>
<td>( D_f )</td>
<td>Fast desensitized state concentration</td>
</tr>
<tr>
<td>( D_s )</td>
<td>Slow desensitized state concentration</td>
</tr>
</tbody>
</table>
Receptors must have at least two binding sites, two open states and two desensitization states. Transitions from the slow desensitized $D_s$ to the fast desensitized state $D_f$ have also been included in subsequent models. These are represented in the seven state model shown in Table 1.

The GABA reaction scheme is modeled by the system of ODE in Table 3, and the model parameters are given in Table 4. At this stage, there is no a priori reason to suspect that the system is stiff, though it seems likely that due to the number of processes present, some may occur on faster timescales than others. We discuss the numerical simulations of this model in Section 2.1.2.

<table>
<thead>
<tr>
<th>$O_1$</th>
<th>First open state concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$O_2$</td>
<td>Second open state concentration</td>
</tr>
</tbody>
</table>

Table 3: GABA reaction scheme equations

<table>
<thead>
<tr>
<th>Equation</th>
<th>Description</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\frac{dR}{dt}$</td>
<td>$= \psi_1(k_{off}, 2k_{on}, R(t))$</td>
<td>2</td>
</tr>
<tr>
<td>$\frac{dN}{dt}$</td>
<td>$= \psi_1(k_{off}, 2k_{on}, R(t)) + \psi_2(2k_{off}, k_{on}, B_1(t)) + pD_s(t)N(t)$</td>
<td>3</td>
</tr>
<tr>
<td>$\frac{dB_1}{dt}$</td>
<td>$= -\psi_1(k_{off}, 2k_{on}, R(t)) + \psi_3(2k_{off}, k_{on}, B_1(t)) - F_1(t) - G_{1s}(t)$</td>
<td>4</td>
</tr>
<tr>
<td>$\frac{dB_2}{dt}$</td>
<td>$= -\psi_2(2k_{off}, k_{on}, B_1(t)) - G_{2f}(t) - F_2(t)$</td>
<td>5</td>
</tr>
<tr>
<td>$\frac{dD_f}{dt}$</td>
<td>$= H(t) + G_{2f}(t)$</td>
<td>6</td>
</tr>
<tr>
<td>$\frac{dD_s}{dt}$</td>
<td>$= -H(t) + G_{1s}(t)$</td>
<td>7</td>
</tr>
<tr>
<td>$\frac{dO_1}{dt}$</td>
<td>$= F_1(t)$</td>
<td>8</td>
</tr>
<tr>
<td>$\frac{dO_2}{dt}$</td>
<td>$= F_2(t)$</td>
<td>9</td>
</tr>
<tr>
<td>$\psi_j(k_1, k_2, f(t))$</td>
<td>$:= k_1B_j(t) - k_2f(t)N(t)$</td>
<td>10</td>
</tr>
</tbody>
</table>
\[ F_j(t) := b_j B_j(t) - a_j O_j(t) \]
\[ G_{ij}(t) := d_{ij} B_j(t) - r_i D_{ij0} \]
\[ H(t) := qD_n(t)N(t) - pD_f(t) \]

Table 4: GABA reaction scheme parameters, in units of per second or per M per second.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a_1)</td>
<td>1100 /s</td>
</tr>
<tr>
<td>(b_1)</td>
<td>200 /s</td>
</tr>
<tr>
<td>(a_2)</td>
<td>142 /s</td>
</tr>
<tr>
<td>(b_2)</td>
<td>2500 /s</td>
</tr>
<tr>
<td>(r_1)</td>
<td>0.2 /s</td>
</tr>
<tr>
<td>(r_2)</td>
<td>25 /s</td>
</tr>
<tr>
<td>(p)</td>
<td>2 /s</td>
</tr>
<tr>
<td>(q)</td>
<td>0.01 /s</td>
</tr>
<tr>
<td>(d_1)</td>
<td>13 s</td>
</tr>
<tr>
<td>(d_2)</td>
<td>1250 /s</td>
</tr>
<tr>
<td>(k_{on})</td>
<td>5x10^6 /M/s</td>
</tr>
<tr>
<td>(k_{off})</td>
<td>131 /M/s</td>
</tr>
</tbody>
</table>

3.2. Numerical stiffness

We shall begin this section with a sample dynamical system that exhibits stiffness, and then seek to convince the audience that merely using higher order or adaptive stepsize algorithms does not alleviate the problem; as long as these methods are explicit. This motivates the discussion on numerical stiffness, and we describe well-known fixes for the problem. Readers who are familiar with the issues of stiffness are encouraged to skip to Section 2.1.4.

To begin with, we are interested in computing the solution \(y(x)\) to the system of ODE

\[ y'(x) = f(x,y(x)), \quad (14) \]
\[ y(a) = \alpha, \ x \in [a,b]. \]

We note that the IVP may be a single equation, or a system. In the latter case the solution \( y(x) \) is a vector. In Henrici’s notation, the recursion used to advance the solution of (14) from \( x_n \) to \( x_{n+1} \) is

\[ y_{n+1} = y_n + h_n \Phi(x_n, y_n, h_n), \tag{15} \]

where \( y_n \) is the numerical approximation to the exact solution \( y(x_n) \). The function \( \Phi \) is called the \textit{increment function} of the method. For example, when \( \Phi(x_n, y_n) = f(x_n, y(x_n)) \), the algorithm obtained is the usual Forward Euler.

\subsection*{3.2.1. Stiffness what is it?}

We begin this section with a simple example to illustrate the phenomenon of “stiffness”.

\textbf{Example 1} \textit{Consider the IVP}

\[ y'' = -10000y - \exp(t) + 10000, \ y(0) = 1, \ \text{te}[0,5]. \tag{16} \]

\textit{The exact solution of this problem is given by}

\[ y(t) = -(1/10001) \exp(t) + 1 + (1/10001) \exp(-10000t). \]

We see from the expression above that the last exponential term \((1/10001)\exp(t)\) should become negligible in comparison to 1. Indeed, when \( t \approx 0.72 \), this term contributes less than \( 1/10^{15} \) to the overall computation.
In order to compare the relative efficiency of various algorithms in the example above, we required that the relative error of the computed solution stayed within $10^{-6}$ during the interval $[0,5]$. For each algorithm, we reduced the step-size or error tolerance until the desired accuracy was achieved. We present the stepsize used, as well as the number of time-steps taken to integrate over the interval $[0,5]$. The algorithms used were:

1. **Forward Euler**: The simplest explicit integrator possible, low-order, and fixed step-length.

2. **ODE23**: MATLAB’s adaptive step-size, second-order algorithm

3. **RK4**: A fixed step-length, explicit fourth-order algorithm

4. **ODE45**: MATLAB’s adaptive step-size, fourth-order algorithm

Table 5: Results of using explicit numerical methods on example 1. The true solution at $t = 5$ is $y(5) = .9851601681...$

<table>
<thead>
<tr>
<th>algorithm</th>
<th>no. of steps</th>
<th>max step</th>
<th>cputime</th>
<th>Y(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Euler</td>
<td>100000</td>
<td>$5 \times 10^{-5}$</td>
<td>339.8</td>
<td>.9851609</td>
</tr>
<tr>
<td>ode23</td>
<td>19902</td>
<td>$3.803 \times 10^{-4}$</td>
<td>67.37</td>
<td>.9851602</td>
</tr>
<tr>
<td>RK4</td>
<td>50000</td>
<td>$1 \times 10^{-4}$</td>
<td>91.31</td>
<td>.9851616</td>
</tr>
<tr>
<td>ode45</td>
<td>60273</td>
<td>$1.088 \times 10^{-4}$</td>
<td>109.41</td>
<td>.9851602</td>
</tr>
</tbody>
</table>

In Table 5, we compile some results using various standard explicit numerical methods.
algorithms. All explicit solvers, with or without variable step-size, work in the same way.

That is, the computation of the approximate solution at $x_{n+1}$ depends on the values of the solution already computed. Each experiment was conducted in MATLAB; on a Linux workstation with a 1.3GHz Athlon processor. The cpu-times reported are in seconds. The algorithms would blow up, yielding NaN, if the step-sizes were much larger. We see that the algorithms are choosing a step-size small enough to accurately resolve the fastest transient.

In other words, the insignificant term $(1/10001)\exp(-10000t)$ governs the choice of step-size!! This is neither a problem of accuracy, nor of adaptivity. The poor performance of all these algorithms on the example points to a deeper issue. Merely using higher and higher order algorithms, even if they are adaptive, does not suffice; the dynamical system 16 exhibits behavior that confounds each of these methods. In particular, all of these algorithms have stability problems; if the step size is not excruciatingly small, the computed solutions “blow up”. We describe this system as stiff.

Heuristically, one useful definition of stiff dynamical systems is: A system in which one or several parts of the solution vary rapidly is characterized as a stiff system; for example, (as an exponential, $\exp(-kx)$ for example, with $k$ large), while other parts of the solution vary much more slowly (as an oscillator, or linearly) is characterized as a stiff system. These are prevalent in the study of damped oscillators, chemical reactions and electrical circuits. Essentially, the derivative or parts thereof change very quickly.
The precise definition of numerical stiffness is difficult to state; however, the phenomenon is one that is widely observed while numerically approximating solutions of differential equations (as seen above). For our purposes, we adopt the following definition of stiffness due to Lambert:

**Definition 1:** If a numerical method with a finite region of absolute stability, applied to a system with any initial conditions, is forced to use in a certain interval of integration a step length which is excessively small in relation to the smoothness of the exact solution in that interval, then the system is said to be **stiff** in that interval.

This definition allows us to concede that the stiffness of a system may vary over the interval of integration. The term ‘excessively small’ requires clarification. In regions where the fast transient is still alive, we expect the step length to remain small. However, in regions where the fast (or slow) transients have died, we would expect the step length to increase to a size reasonable to the problem.

A “cure” for stiffness is the use of implicit algorithms. These are algorithms where the approximation $Y_{n+1}$ to the true solution $y$ at $t_n + \frac{1}{h}$ is given by

$$Y_{n+1} = Y_n + h\Phi(Y_{n+1}, t),$$

where $\Phi$ is chosen according to the system being solved, the accuracy desired, etc. Note that the update $Y_{n+1}$ is now the solution of a (typically) non-linear system. Thus, each
step of our algorithm requires a nonlinear solve, an expensive proposition. However, since the domain of stability of implicit algorithms is much larger than that of explicit algorithms, most state-of-the-art stiff solvers employ adaptive implicit algorithms. For example, the built-in MATLAB solvers ode23s and ode23tb are implicit Runge-Kutta methods with variable step size control. The savings in terms of computational time is well worth the effort of solving the nonlinear problem. To drive this message home, let us return to the example we began this section with, and use a

1. **Backward Euler:** the implicit analog of Forward Euler, this method requires a fixed step-size.

2. **MATLAB’s ODE23s:** this method uses an adaptive step-size, and is a second-order algorithm.

Table 6: Some implicit algorithms used to compute example 1. Compare these with similar explicit algorithms in Table 2.1.3.1.

<table>
<thead>
<tr>
<th>algorithm</th>
<th>no. of steps</th>
<th>max step</th>
<th>Cpu-time</th>
<th>Y(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Backward</td>
<td>2500</td>
<td>.002</td>
<td>.11</td>
<td>.9851898</td>
</tr>
<tr>
<td>Euler</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ode23s</td>
<td>179</td>
<td>0.1094</td>
<td>0.61</td>
<td>0.9851601</td>
</tr>
</tbody>
</table>
Table 6 summarizes the performance of these implicit algorithms. Despite the nonlinear solve, these algorithms outperform those in Table 5 by a couple of orders of magnitude both in terms of number of time-steps, and cpu-time taken.

### 3.3 The GABA reaction scheme

These numerical experiments were conducted after we had computationally studied a completely unrelated system: the Santillán-Mackey model of gene transcription. The latter was a stiff-delay differential system of 4 reactants, and we found invariant manifolds present in the underlying dynamics. To our surprise, our numerical investigations of the GABA reactions led us to observe similar invariants. In locating invariant manifolds in the GABA system, we relied upon observations from our previous studies.

We typically ran the GABA model with the initial conditions in Table 7. When describing experiments, we shall specify the initial GABA concentration, $N_0$.

Table 7: Initial conditions for the GABA scheme

<table>
<thead>
<tr>
<th>$R_0$</th>
<th>$1 \times 10^{-6}$ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_0$</td>
<td>$(0.5, 4096) \times 10^{-6}$ M</td>
</tr>
<tr>
<td>All other initial variables set to zero</td>
<td></td>
</tr>
</tbody>
</table>
3.3.1 Stiffness in the GABA reaction scheme

We first ran ode45 on the GABA system. We set the initial GABA concentration to $N_0 = 5 \times 10^{-7}$M and ran the experiment for $t$ between 0 and 1 second. We found ode45 to be unstable. It starts the integration with a step length of $2.5 \times 10^{-4}$s. Neither setting the initial step size to $1.0 \times 10^{-4}$s, nor the maximum step size to $1.0 \times 10^{-3}$s, makes the solution stable. With a maximum step size of $5 \times 10^{-4}$s, ode45 integrates the system well in 2000 steps. With smaller step sizes, the plots are graphically indistinguishable at this resolution. With ode23s, we find good convergence with the maximum step length set to 0.01s. This step length is used by ode23s throughout the interval, completing the integration in 100 steps, a twenty-fold improvement over ode45.

Invariant manifolds were discovered in the GABA model. Since there are eight dependent variables, there are 28 possible two-dimensional phase space plots and 56 possible three-dimensional phase space plots. With this alone, it would be quite tedious to identify invariant manifolds. We noticed that the time plots for the variables, which became linearly related on the invariant manifolds of the Santillán-Mackey system, closely resembled each other qualitatively. Using this as a starting point, we plotted the variables whose time plots looked qualitatively similar in the GABA
scheme. To our surprise, we found two invariant manifolds again described by linear relationships between the variables. In Figure 1 we see the variables B1 and O1 pair up. The relationship is given approximately by $O_1 \approx 0.182B_1$. The solution reaches this manifold on the order of $10^2$ seconds. The variables $B_2$, $O_2$ and $D_1$ also become dependent, as seen in Figure 2. The relationship here is given approximately by $D_1 \approx 48.4$, $B_2 \approx 2.73O_2$. The solution reaches this manifold on the order of tenths of seconds (around 0.3s-0.5s). In all, the system goes from eight dimensions to seven on the order of 1/100 seconds, and then down to dimension five, tenths of seconds later. An exhaustive search in the remaining variables reveals that these are the only invariant manifolds the GABA reaction system. As for the Santillán-Mackey model, these invariant manifold observations provide a key experimental test of the model.

3.3.2 Role of desensitization in generating inhibitory currents.

The rate at which the receptor enters the desensitization state will affect the shape of inhibitory currents, and this may be a means by which endogenous enzymes regulate receptor activation. Desensitization tends to prolong the inhibitory current and keeps the transmitter in the bound state of the receptor. This would be expected to affect output of neuronal circuits. GABA inhibitory currents are known to be important in the generation of synchronized oscillations in the cortex, thalamus and hippocampus. Low frequency
rhythms in the neocortex range from 5-10 Hz (0.1-0.2 s) coinciding with the appearance of the lower dimensional manifolds in the GABA reaction scheme. The linear coupling of the bound, desensitized and open states of the receptor between 0.1 - 0.5 s occurs at a physiologically relevant timescale to affect timing of neural circuit activity. This suggests that simple relationships may exist between the states of the receptor at low frequencies of receptor activation. The number of channel openings will be directly proportional to the amount of transmitter traversing through the bound and desensitization states. So, if the transmitter is rapidly removed some of it will still be bound to the receptor flipping through bound, open and desensitized states, and the amount buffered will be directly proportional to the receptor in the open state just prior to the free GABA removal. Therefore, the system will have some memory of the activity at the point free GABA is removed. The existence of such a simple linear buffer may have important implications for the transmission of information through the GABA receptor. Such a buffer may aid in the routing of signals from one neuron to another. To investigate this possibility we are now building a model in which pulses of GABA are delivered to the receptor. We will monitor the amount of GABA buffered and to see if this is related to the accurate transfer of frequency information through the receptor.

It is experimentally possible to deliver high concentration pulses of GABA onto neurons and measure the inhibitory potentials that are generated. A range of frequencies could be delivered to the cell with the GABA receptors while measuring the fidelity of
the response using information theoretic approaches. These responses can be compared to the effect of GABA in the presence of drugs that change the kinetic parameters in the GABA model. The quantitative relationship between the relative states of the receptor and frequency transfer can then be used to assess the role of desensitization state in information processing.

3.4. Conclusion.

The reaction scheme of GABA interacting with the ligand-gated ion-channel demonstrates numerical stiffness. We compared various explicit and implicit numerical methods to solve for the reaction scheme, and found implicit methods (Backward Euler, ode23s) performed orders of magnitude better than explicit methods (Forward Euler, ode23, RK4, ode45) in terms of step size required for stability, number of steps and cpu time. Interestingly, we observed the existence of low dimensional invariant manifolds upon solving the system of 8 ordinary differential equations for the GABA reaction scheme. The emergence of simple direct relationships between the receptor states may have some important roles in biological signal transmission.

4.1 Introduction.

The predominant mechanism of signal transmission between neurons is through the release of chemical signals that act upon specialized receptors to evoke changes in the electrical and biochemical status of the postsynaptic neuron. One of the first constraints in interpreting the received signal arises from the binding and unbinding rate constants of the transmitter with stoichiometries greater than one to its multiple binding of ligands to a single receptor. These binding interactions occur under rapidly changing transmitter concentrations at non-equilibrium conditions. Experimentally reproducing such changes, even in fast perfusion experiments, has proven to be extremely difficult. In order to guide our studies of these processes we have sought to model receptor activation and deactivation using methods that do not rely on equilibrium conditions or assumptions about initial conditions. We developed a modeling approach that simulates the binding of the transmitter to a receptor even under pulsatile and noisy transmitter conditions. Fire-integrate models that utilize inputs using fractional-gaussian-noise-driven Poisson processes produce realistic output spike trains and inputs with long-range dependence similar to that found in most subcortical neurons in sensory pathways. To study the transfer of signals through this system we examined the responses of the receptor to
complex and noisy inputs using Fourier analysis. A rate code in neuronal responses can be characterized by its time-varying mean firing rate and describes neural responses in many systems. The noise in rate coding neurons has been quantified by the coherence function or the correlation. Unbiased estimators for the measures of signal to noise ratios in neuronal responses have been used to measure inherent noise in classes of stimulus-response models that assume that the mean firing rate captures all the information. We have extended the use of signal to noise measurements to determine information processing capabilities of chemical neuronal transmission in the presence of psychoactive drugs.

This approach will lead to better understanding of the relationships between the kinetic properties of the receptor and the information transfer functions of the receptor in a noisy environment. Also the predictions from the mathematical modeling approach can be used to test the role of a particular receptor subtype in information processing in experiments performed in-vivo. Development of non-linear mathematical models will aid in more fully understanding drug mode of interaction for psychoactive drugs.

4.2 Modeling approach.

Receptor mediated gating of ion channels can be expressed as a set of bimolecular
interactions that include state transitions. Our approach was to develop analytical formalisms that describe and predict receptor interactions with no assumption of steady state. One important requirement was to solve for the kinetics of bimolecular interactions when there is depletion of free ligand. This requirement allows changes in bound receptor to be modeled at any initial ligand and receptor concentration. It also allows experiments to be designed in which binding occurs at low ligand and high receptor concentrations. Under these conditions the forward rate of the bimolecular interaction is slowed down relative to the reverse rate, and important changes in early kinetics of receptor interactions can be detected. The kinetic courses for many reactions (opposing unimolecular, bimolecular, termolecular) have been previously described using analytic solutions. In our modification, the general solution includes an expression for the initial concentration of bound receptor (required for solving difference equations), and multiple ligand binding to a receptor. To model the subsequent transitions to activated states of the receptor, our approach has been to use the exact solutions to describe all the possible kinetic interactions and then solve the variables using difference equations. Simulations can be performed under ligand depleting conditions and noisy environments that are likely to occur in the synapse.

4.2.1 Analytic expressions for Stoichiometric interactions.
Since ligand-gated ion-channels are composed of multiple subunits they can potentially bind multiple ligands, and, given the importance of agonist receptor interactions in shaping the postsynaptic response we have now developed a more general model to describe binding kinetics for ligand-gated ion channels that have multiple binding sites. We use binding to GABA$_A$ receptors to show how these analytic solutions can be used to solve for stoichiometry of ligand interaction. This novel analytical formalism will allow for the determination of agonist affinities without prior knowledge of the Markov kinetic scheme at low ligand and high receptor concentrations.

\[
\begin{align*}
    x_0 + y & \xrightarrow{2K/L} x_1 \\
    x_1 + y & \xleftarrow{K/2L} x_2
\end{align*}
\]

\textbf{K}: on rate  \\
\textbf{L}: off rate  \\
\textbf{x}_0: \text{free receptor}  \\
\textbf{x}_1: \text{single ligand bound}  \\
\textbf{x}_2: \text{two ligands bound}  \\
\textbf{y}: \text{free ligand}

Four differential equations describe the binding of two ligands to a receptor:

\[
\frac{d}{dt}x_0 = -2 \cdot K \cdot x_0 \cdot y + L \cdot x_1
\]
Let:

\[
\frac{d}{dt} x_1 = 2 \cdot K \cdot x_0 \cdot y - K \cdot x_1 \cdot y - L \cdot x_1 + 2 \cdot L \cdot x_2
\]

\[
\frac{d}{dt} x_2 = K \cdot x_1 \cdot y - 2 \cdot L \cdot x_2
\]

\[
\frac{d}{dt} y = -2 \cdot K \cdot x_0 \cdot y - K \cdot x_1 \cdot y + L \cdot x_1 + 2 \cdot L \cdot x_2
\]

Let:

Express the differential of \( u \) in terms of \( x_0, x_1, \) and \( x_2 \)

\[
\frac{d}{dt} u = 2 \cdot \frac{d}{dt} x_0 + \frac{d}{dt} x_1
\]

\[
\frac{d}{dt} u = 2 \left( -2 \cdot K \cdot x_0 \cdot y + L \cdot x_1 \right) + \left( 2 \cdot K \cdot x_0 \cdot y - K \cdot x_1 \cdot y - L \cdot x_1 + 2 \cdot L \cdot x_2 \right)
\]

\[
\frac{d}{dt} u = -2 \cdot K \cdot x_1 \cdot y - K \cdot x_1 \cdot y + L \cdot x_1 + 2 \cdot L \cdot x_2
\]
\[
\frac{d}{dt} u = \frac{d}{dt} y
\]

Re-express in terms of initial conditions:

\[y_0 - y = u_0 - u\]
\[u = u_0 - y_0 + y\]

Express the differential of \(v\) in terms of \(x_0, x_1, x_2\):

\[
\frac{d}{dt} v = \frac{d}{dt} x_1 + 2 \cdot \frac{d}{dt} x_2
\]

\[
\frac{d}{dt} v = 2K \cdot x_0 \cdot y - K \cdot x_1 \cdot y - L \cdot x_1 + 2L \cdot x_2 + 2 \left( K \cdot x_1 \cdot y - 2L \cdot x_2 \right)
\]

\[
\frac{d}{dt} v = 2K \cdot x_0 \cdot y + K \cdot x_1 \cdot y - L \cdot x_1 - 2L \cdot x_2
\]

\[
\frac{d}{dt} v = -\frac{d}{dt} y
\]

Re-express \(v\) in terms of initial conditions:

\[y_0 - y = -v_0 + v\]
\[v = v_0 + y_0 - y\]

Express differential of \(y\) in terms of \(u\) and \(v\):
\[ \frac{dy}{dt} = -K \cdot y \cdot u + L \cdot v \]

Substitute \( u \) and \( v \) into differential of \( y \):

\[ \frac{dy}{dt} = -K \cdot y \left( u_0 - y_0 + y \right) + L \left( y_0 - y + v_0 \right) \]

\[ \frac{dy}{dt} = -K \cdot y \cdot u_0 + K \cdot y \cdot y_0 - K \cdot y^2 + L \cdot y_0 - L \cdot y + L \cdot v_0 \]

Integrate differential of \( y \) in terms of polynomial coefficients \( a \), \( b \) and \( c \) with roots \( y_1 \) and \( y_2 \):

\[ a = -K \quad b = -K \cdot u_0 + K \cdot y_0 - L \quad c = L \cdot y_0 + L \cdot v_0 \]

\[ y_1 = \frac{1}{2 \cdot a} \left( -b + \sqrt{b^2 - 4 \cdot a \cdot c} \right) \]

\[ y_2 = \frac{1}{2 \cdot a} \left( -b - \sqrt{b^2 - 4 \cdot a \cdot c} \right) \]

Integral solves for ‘\( y \)’ at time ‘\( t \)’, \( y_t \):

\[ y_t = \frac{y_1 - \left( \frac{y_2 - y_1}{y_0 - y_2} \right) e^{-K(y_1 - y_2) \cdot t}}{1 - \left( \frac{y_2 - y_1}{y_0 - y_2} \right) e^{-K(y_1 - y_2) \cdot t}} \]

We derived a novel solution for ligand-receptor interaction with stoichiometry greater than 1 even under ligand depleting conditions. Using the appropriate
substitutions, the free ligand concentration over time was determined with multiple ligand interactions with a single receptor. By making substitutions for the number of ligand free sites on the receptor (\(u\)) and ligand occupied sites of the receptor (\(v\)), direct relationships are described between free ligand levels (\(y\)) and the substituted parameters ‘\(u\)’ and ‘\(v\)’. The level of free ligand was described by an exact solution in terms of the rate constants, initial \(u_0\), \(v_0\) and initial free ligand concentration. The derivation is shown for 2 ligand binding sites for one receptor. The same method was used to solve for up to five ligands binding to a receptor protein (\(dy/dt = du/dt\), \(dy/dt = -du/dt\) for all cases).

We have analytically solved the differential equations that describe the binding of up to five ligands to a receptor with non-interacting, homogenous binding sites. When the initial ligand concentration is much greater than the initial free receptor concentration the amount of bound receptor is linearly related to the stoichiometry at all receptor concentrations. Consequently the curve at high stoichiometry is a scaled version of the curve at low stoichiometry (Figure 3A). However, at low initial ligand concentrations, when there is measurable ligand depletion, the relationship is markedly curvilinear (Figure 3B).

The binding of agonists to multiple sites on a receptor is a difficult process to study, often requiring technically intensive methods. For example, the binding of glycine and glutamate to the tetrameric NMDA receptor has been examined by co-expressing wild type and low-affinity (or dominant negative mutant) subunits in heterologous cells at different ratios. The observed shifts in affinity at different subunit expression ratios were consistent with two binding sites for glutamate and two for glycine. Another approach examined the stoichiometry of nAChRs using a subtype specific toxin (MLA) to block acetylcholine responses. Recovery from inhibition showed complex kinetics that was solved as a system of first order differential equations for binding of multiple inhibitor molecules per receptor. In this linear chain model, the ligand concentration was assumed
to be a constant, and binding of a single molecule of MLA to the receptor prevented the functional response to ACh. The model predicted the observed S-shape of the recovery with five putative binding sites for MLA.

The analytical solution for determining stoichiometry described in the present paper is similar to that of Palma et al (1996), except that we do not make any assumptions about ligand depletion. This has the advantage that a wider range of ligands with lower affinities can be used, and the number of agonist binding sites can be assessed directly without using mutant expression or subtype specific toxins. For example, at very high ligand concentrations the binding reaction is simply scaled by the number of sites on a receptor, hence the stoichiometry can be calculated by comparing binding at low initial ligand concentrations (Figure 3B) and after adding excess ligand (Figure 3A). Any differences in the apparent stoichiometry between the two methods would begin to unmask important cooperative interactions between the agonist binding sites.

4.2.2. Bimolacular Interactions.

Current methods for estimating agonist affinity require detailed knowledge of the kinetic scheme and estimates of the microscopic binding transitions; these are often derived from fast agonist application to outside out membrane patches. Such Markovian schemes can be complicated (e.g., for the GABA_A receptor, two binding sites for the agonist, two open states and two desensitization states although the kinetics of the receptor states usually can be solved numerically.) Such studies reveal that agonist binding rates can be orders of magnitude slower than free diffusion, indicating a ligand specific energy barrier between receptor bound states. These energy barriers may be responsible for agonist selectivity. Unfortunately, details of the receptor activation and desensitization states are not available for all receptor types. Therefore, we see a great use for equations that determine agonist on and off rates without prior knowledge of the kinetic scheme. Previously we described an analytical method to solve for binding
reactions that do not assume equilibrium conditions or a particular state model, so
binding at early time points can be used to determine the affinity of the agonist to the
receptor. The utility being that by controlling the time of reaction and initial ligand
concentrations, most of the measured binding is with the receptor before any further
receptor transitions or interactions with other proteins. This simplified procedure will
allow for the screening of many compounds to quickly elucidate structure activity
relationships between receptors and agonists. The bimolecular solution is presented
below:

\[
\frac{dRN}{dt} = a \cdot RN^2 + b \cdot RN + c
\]

\[a = K\]

\[b = -K(2 \cdot RN + R + N) - L\]

\[c = K(RN_t^2 + RN \cdot R + N \cdot RN_t + N_t \cdot R_t)\]

\[z1 = \frac{- b + (b^2 - 4 \cdot a \cdot c)^{1/2}}{2 \cdot a}\]

\[z2 = \frac{- b - (b^2 - 4 \cdot a \cdot c)^{1/2}}{2 \cdot a}\]
\[ E = e^{(\Delta t \cdot a \cdot (z_2 - z_1))} \]

\[ \text{R}_n_{t+1} = \frac{z_2 \cdot z_1 - z_2 \cdot \text{R}_n_{t} + E \cdot z_1 \cdot \text{R}_n_{t} - E \cdot z_1 \cdot z_2}{z_1 \cdot -\text{R}_n_{t} + E \cdot \text{R}_n_{t} - E \cdot z_2} \]

\[ \text{Changebound} (K, L, \Delta t, R_t, L_t, \text{R}_n_t) = \text{R}_n_{t+1} - \text{R}_n_t \]

The exact solutions for bimolecular interactions were derived using only the assumptions of mass action and the conservation of mass. The rate of appearance of the bound form of the receptor (\( \text{R}_n \)) was expressed as a differential equation in terms of receptor concentration, ligand concentration and the on (K), and the off (L). This differential resulted in a polynomial that had a standard solution, which describes the formation of the bound receptor \( \text{R}_n \) at time \( t+1 \) (\( \Delta t \) increment) given ligand, receptor and bound receptor concentrations at time \( t \). The change in the bound concentration ‘\( \text{R}_n \)’ from time ‘\( t \)’ to ‘\( t+1 \)’ is given by the function Changebound. The appearance of 1 mole of the bound form results in the disappearance of 1 mole of receptor and 1 mole of ligand. Therefore, the concentrations of \( R_N \) and \( \text{R}_n \) can all be calculated at time ‘\( t+1 \)’ using the function Changebound.

\[ \text{4.2.3. Receptor transition.} \]
\[
\frac{dRR}{dt} = K_f \cdot R - K_r \cdot RR
\]

\[E2 = e^{(\Delta t \cdot (K_r - K_f))}\]

\[
\text{Changetrans}(K_f, K_r, \Delta t, R_t, R_{Rt}) = R_{Rt+1} - R_t
\]

If a receptor (R) transitions to a new state (RR), the forward and reverse rates are functions of the concentration of each receptor state and the rate constants. The function Changetrans describes the change in the concentration of RR at ‘t+1’ (time increment \(\Delta t\)) given concentrations of R and RR at time ‘t’. The two functions Changebound and Changetrans were used to solve for the GABA scheme outlined below.

4.3 Using difference equations to model complex kinetic schemes: for GABA scheme:

\[
\text{ChangeB1} = \text{Changebound}(2 \cdot k_{on}, k_{off}, \Delta t, R_t, N_t, B_{1t})
\]

\[
\text{ChangeB2} = \text{Changebound}(k_{on}, 2 \cdot k_{off}, \Delta t, B_{1t}, N_t, B_{2t})
\]
\[
\text{ChangeDf} = \text{Changebound} (q, p, \Delta t, Ds, N, Df)
\]

\[
\text{ChangeO1} = \text{Changetrans} (b1, a1, \Delta t, B1, D1)
\]

\[
\text{ChangeDs} = \text{Changetrans} (d1, r1, \Delta t, B1, Ds)
\]

\[
\text{ChangeO2} = \text{Changetrans} (b2, a2, \Delta t, B2, O2)
\]

\[
\text{ChangeDfII} = \text{Changetrans} (d2, r2, \Delta t, B2, Df)
\]

\[
R_{t+1} = R_t - \text{ChangeB1}
\]

\[
N_{t+1} = N_t - \text{ChangeB1} - \text{ChangeB2} - \text{ChangeDf}
\]

\[
B1_{t+1} = B1_t - \text{ChangeDs} - \text{ChangeO1} - \text{ChangeB2}
\]

\[
B2_{t+1} = B2_t + \text{ChangeB2} - \text{ChangeO2} - \text{ChangeDfII}
\]

\[
Df_{t+1} = Df_t + \text{ChangeDf} + \text{ChangeDfII}
\]

\[
Ds_{t+1} = Ds_t - \text{ChangeDf} + \text{ChangeDs}
\]

\[
O1_{t+1} = O1_t + \text{ChangeO1}
\]

\[
O2_{t+1} = O2_t + \text{ChangeO2}
\]

Each kinetic scheme was reduced to its component reactions, which included bimolecular interactions and receptor state transitions. Because each type of reaction has an analytical solution, the reactions were solved as the corresponding set of difference equations. The scheme for GABA included three binding reactions and four state
transitions. Functions for the binding reactions (Changebound) and state transitions (Changetrans) described the change in concentrations of the bound states of the receptor from time ‘t’ to ‘t+1’. Difference equations constructed from the functions were used to calculate the concentrations of free ligand (N), free receptor (R), open states, bound states, and desensitized states of the receptor at each time point. The kinetic parameters for the difference equations were the same as for differential equations in Table 4 (receptor concentration = 10nM).

4.4. Waveform analysis.

4.4.1 Power spectra.

Synaptic "noise" is caused by the nearly random release of transmitter from thousands of synapses. Power spectral density can be used to analyze synaptic noise and deduce properties of the underlying synaptic inputs. Delta kinetic models examine classes of analytically solvable kinetic models for synaptic activation of ion channels. These models show that for this class of kinetic models an analytic expression for the power spectral density to derive stochastic models with only a few variables and can be solved for analytic expressions of the Vm distribution that covers parameters values several orders of magnitude around physiologically realistic values. The power spectra provides for model building using intracellular recordings in vivo that includes spectral
structure of synaptic noise. We used this method to describe receptor activation and tuning properties in a physiological setting. We performed simulations for heterosynaptic regulation of GABA release in the cerebellum described. Transmitter pulses were Poisson distributed with mean frequencies of 50 Hz for evoked signals and 12 Hz for presynaptic background signal with white noise distributed amplitude of 1.5mM. The power of the response at frequency bands of up to 150 Hz was measured for 25 simulations (± SEM). Each train had a mean frequency of 50 Hz with or without the 12Hz presynaptic background signal. Simulations were performed using discretized time points of 100 µs. Each pulse train and the corresponding response profile were expressed in the frequency domain for evoked signals or evoked plus presynaptic background signals. The underlying frequencies of a waveform can be expressed as the sum of functions of the form A.sin(2.pi.f.t) and B.cos(2.pi.f.t). The real numbers in the time domain returned coefficients that are imaginary numbers in the frequency domain. Discrete Fourier Transform (fft) of signal (x) at sampled time ‘t’ is estimated by:

\[
\text{fft}(x)_t = \frac{1}{\sqrt{N}} \sum_{m} x_m \exp \left\{ 2 \pi i \frac{t - \tau}{N} \cdot \mu \right\}
\]

Where N is the total number of data points divided into ‘m’ elements.

The transformed values for GABA levels (N) and open state conformation (O (O1 +
O2)) are expressed as complex values in frequency space (f):

\[ N_m(f) = \text{fft}(N)_t \]

\[ O_m(f) = \text{fft}(O)_t \]

The squared magnitude of the Fourier transform is a relative measure of the representation of a frequency in the waveform (power spectrum at frequency f).

\[ \text{Power}_{N_m}(f) = \left| N_m(f) \right|^2 \]

\[ \text{Power}_{O_m}(f) = \left| O_m(f) \right|^2 \]

### 4.4.2 Coherence.

The representation of a frequency in two waveforms (the input signal and a response profile of the two open states of the GABA\(_A\) receptor) can be measured using a coherence function. A value of one indicates a strong, noise-free, linear dependence of the response on the input signal in that frequency band.

\[ \text{Coherence} (f) = \frac{\left| N_m(f)O_m(f)^* \right|^2}{\text{Power}_{N_m}(f)\text{Power}_{O_m}(f)} \]

The function at each frequency band (f) is the squared magnitude of the cross spectrum (the * denotes the complex conjugate) of the two signals divided by the power spectrum of the input signal multiplied by the response.

### 4.4.3 Signal to Noise Ratio.
Dividing the coherence by one minus the coherence at a frequency band gives the signal to noise ratio, which is proportional to the ratio of the strengths of the signal and the strength of the noise. If there is strong representation above noise of any frequency band in the input and signal waveforms, then values greater than one will be observed.

The original time series of 16384 data points (10,000 Hz; 1.6384sec) was broken into 10 segments that overlapped by 70%. Each segment was Fourier transformed, and the coefficients of the transformed magnitudes were averaged over the 10 regions. The length of this segment corresponded well with the scale of the frequency bands that were being analyzed, and it gave a good estimate of the magnitude at each frequency band from the original time series. There was a trade-off between obtaining good estimates and detecting signals with low frequencies. All calculations were performed using Mathcad (Mathworks).

5. Modeling complex receptor interactions.

The analytical equations described here can also be used to model complex receptor kinetics. In this approach, the analytical solutions are incorporated into sets of simultaneous difference equations that describe occupancy vectors at any time point given initial conditions using difference equations for schemes such as the interactions
with the GABA_A receptor. Both short-term (receptor transitions) and long-term modulatory events (desensitization, associations with other proteins and phosphorylation) can be modeled.

As a test system we have concentrated our analysis on the GABA_A receptor. Although the subunit composition of GABA_A receptors in the brain is not clear, we have used a seven state model in our simulations. The seven state model is the minimum required to predict GABA responses and to reconcile hippocampal macropatch and single channel recordings. These state transitions can be described by a set of ordinary differential equations, which can be solved numerically. However, these solutions are limited to simple inputs (i.e., transmitter levels have to be square waves, sine waves or some other differentiable function). In contrast, the difference equation method achieves similar results for a single long pulse of GABA, but it can be extended to simulate the response of a receptor to noisy inputs. The method of solving simultaneous difference equations does not require complicated numerical integration techniques and can be programmed in any spreadsheet. This has potential for being developed into “web browser” applications to compare predictions from computer simulations with experimental data.

5.1 **Modeling receptor activation during changing transmitter levels.**

One of the major goals accomplished by this analytical approach was the development of a model that predicts how complex continuous signals of any waveform might drive multistate receptors.

Previous attempts to describe activation of ligand gated ion channels in brain synapses approximated the time course of a transmitter signal with a sequence of time steps. At each time point the occupancy of different states of the receptor was calculated using analytic functions derived by solving the Q-matrix for an n-state kinetic scheme. The results presented in the current paper suggest that receptor activation and transition
between multiple states can be determined at each time step using difference equations. For example, it has been shown empirically that during GABA binding, fast entry into the desensitization state, coupled to fast recovery from desensitization and reopening of channels, results in a prolonged inhibitory post synaptic current. In our simulations, instantaneous delivery of GABA followed by its rapid clearance results in prolonged activation of the receptor.

Because rapid changes in GABA levels have such important consequences for signaling, it is important to estimate how they influence receptor states. This is difficult to determine experimentally where the synaptic release and clearance of GABA (approx 100 microsec) appears to be about an order of magnitude quicker than fast perfusion systems (3 mM GABA delivered for 1 msec). The methods described here provide a way to predict the effects of very fast complex pulses on the receptor state from the responses measured during slower activation. This in turn will be very important for understanding the dynamics of transmitter levels in the synaptic cleft.

Previous studies showed the information transfer properties of changing GABA levels by simulating a train of agonist pulses in the synapse; a Poisson distribution specified the time at which the pulse occurred, and at that time point the level of the agonist rose to between 1 and 5 mM according to a white noise distribution. The agonist either bound to the receptor in the free or monoliganded state; or was cleared by uptake and diffusion. The clearance was approximated by a double exponential decay (2311 and 28 sec\(^{-1}\)). Each train had a mean pulse frequency (the driving frequency) over the 1.6384 sec period of 10, 15, 20, 30 or 50 Hz. At each driving frequency receptor activation was
simulated using 49 trains.

These pulse trains were used in the simulated binding reaction to examine the dynamics of the GABA_A receptor at different driving frequencies. At 10Hz there was a large initial response to the first pulse, but the response declines progressively. This is mostly due to the build up of the desensitized state of the receptor. At low driving frequencies (e.g., 10Hz) any responses that exceeded 5Hz had signal-to-noise ratios greater than 1. Therefore, in a single train of Poisson distributed pulses any signals greater than 5Hz are better represented in the response profile. There were also two peaks (frequencies that were maximally represented in the response profile) at 90 Hz and 120 Hz when the driving frequency was 10Hz, suggesting that at low background activity the receptors are ‘tuned’ to receive stimulation frequencies corresponding to gamma waves.

At higher driving frequencies the signal to noise ratios were reduced (with the exception of the driving frequencies which peak because of the increased number of stimulation pulses). In fact, at driving frequencies greater than 20 Hz the signal to noise ratios were less than one, suggesting that the noise is greater than the signal. Interestingly, there was also a time dependent change in the representation of the signal in a response profile. The signal was better represented in the second half of the GABA response even though the peak amplitudes in the later segment were much reduced. This increased response to stimulation results from an accumulation of the desensitization state of the receptor and suggests desensitization might have an important role in filtering information at the synapse.

5.2 The effect of psycho-active drugs on information transfer.

To test the potential effect of drugs on signal transfer through the GABA_A receptor,
simulations were performed in which the rate constants $k_{on}$, $k_{off}$, and $d_2$ were modified to values estimated from empirical data. Compared to the responses to GABA alone, both the low affinity agonist THIP ($k_{off} = 1125 \text{ sec}^{-1}$) and the GABA$_A$ modulator, chlorpromazine ($k_{on} = 5 \times 10^5 \text{ M}^{-1}\text{sec}^{-1}$, $k_{off} = 400 \text{ sec}^{-1}$) allowed consistent responses to be maintained throughout the pulse train. However, responses to THIP were of large amplitude, and those to chlorpromazine were small compared to GABA alone. In contrast, GABA stimulation in the presence of the neurosteroid pregnenolone (which causes an increased rate of entry into the desensitization state, $d_2 = 4750 \text{ sec}^{-1}$) evoked small peak responses that rapidly declined over time.

In all these examples, as with GABA alone, increasing the driving frequency decreased the signal to noise ratios at all response frequencies. However, these drugs also affected the signal to noise ratio of the receptor in specific ways. Modifying $k_{on}$ and $k_{off}$ at 10Hz driving frequency (using chlorpromazine and THIP) produced high signal to noise ratios with chlorpromazine being particularly efficacious. As the driving frequency increased to 20 and 50 Hz the difference in the signal to noise ratios between chlorpromazine and THIP was reduced at all frequencies. Pregnenolone completely inhibited the transfer of information at all frequencies, suggesting that entry into the desensitization state can have an even more profound influence on receptor signaling than changes in the $k_{on}$ and $k_{off}$ rates.

By modeling receptor/ligand interactions under non-equilibrium conditions it is possible to predict the effects of receptor state on the transfer of a signal through the receptor. As an initial demonstration of this capability we have used the signal to noise ratio as a simple measure of information transmittance. We show that GABA signals are likely to be filtered in a time dependent manner. Initially, when entry into the desensitized state is fast, the peak response to GABA is high but the signal to noise ratio is low (suggesting that frequency information is lost), whilst at later time points the peak responses are reduced and the signal to noise ratios are higher. For these longer duration
trains more frequency information is passed through the opening of the channel if downstream cellular processes can detect small amplitude responses. Because of this important role of desensitization, the subunit composition and phosphorylation state of a receptor will strongly influence its frequency processing properties. The modeling presented here shows transmittance of signals at 90Hz (SNR=2.56±0.252) and 120Hz (SNR=2.68±0.221) can be favored at low driving frequencies (10Hz). This raises an interesting possibility that a large population of neurons firing at low frequencies will favor transmittance of information at higher frequencies corresponding to gamma type oscillations. The microscopic properties of the receptor itself may augment the oscillations generated by the neuronal circuitary.

This method can also be used to predict the effects of pharmacological agents on signal transfer. For example, our simulations predict that when there is a noisy input pregnenolone markedly decreases the signal to noise ratio at all frequency bands and has a strong inhibitory effect on the GABA response. It would be interesting to see how this property relates to the cognitive enhancing effects that have been described during pregnenolone treatment. This response contrasts with the effect of chlorpromazine, which modifies GABA transmission by changing the agonist binding and unbinding rates. This drug increases information transfer at all frequencies but as the driving frequency is increased the signal to noise ratio decreases compared to GABA alone. Consequently, by changing binding rates chlorpromazine serves to filter high frequency inputs. At higher driving frequencies (50Hz) information transfer at lower frequencies (<50Hz) is more favored than at higher frequencies. Therefore, drugs like chlorpromazine and THIP may aid the transition to beta type oscillations.

Because these results can be generated in noisy and complex stimulus conditions, predictions can be tested in a relatively intact functioning preparations. It is hoped that these modeling methods will lead to important insights into the role of transmitter binding and unbinding kinetics in synchronizing and tuning receptors and how
pharmacological agents disrupt such signaling. The importance of noise in the neuronal transmission was investigated in simulation studies using uncoupled Hodgkin-Huxley model neurons stimulated with sub-threshold signals. The results showed improvements in both the degree of synchronization among neurons and the spike timing precision, suggesting that the nervous system is capable of exploiting temporal patterns to convey more information than just using rate codes.

5.3 Model for heterosynaptic modulation of synaptic release.

Neuronal plasticity underlies complex behaviors like learning and memory. One important mechanism by which activity dependent changes occur is through heterosynaptic modulation, in which an axo-axonal synapse acts upon the presynaptic terminal to either facilitate or inhibit vesicle release. Presynaptic neuromodulators have shown to significantly change the responses during low frequency spike trains in various brain preparations suggesting an important role for this mechanism in neuronal function. Neurotransmitter action on presynaptic receptors is likely to occur in a background of continuous and noisy activity. Previously, we simulated GABA_A receptor ion channel activation using exact solutions for simple bimolecular interactions and receptor transitions in sets of difference equations. Because it is applicable to noisy systems, we used the difference method to investigate the frequency information processing capabilities of GABA_A receptors and predicted how pharmacological agents may modify these properties. We have now extended these studies to investigate the role of
heterosynaptic modulation of the frequency information using the Cerebellar Purkinje
cells as a model system and applied this approach to analyze the transfer properties of a
receptor when the primary signal is modulated by presynaptic background activity.

5.3.1. Modulation of the Cerebellar synapse by inhibitory neurons.

We concentrated our modeling efforts on the synaptic junction between
basket/stellate cells and the Purkinje neuron in the cerebellum. Studies have
hypothesized the presence of presynaptic glutamate receptors (NMDA) on the
presynaptic terminal of the basket/stellate cell. Addition of NMDA to the molecular
layer of the cerebellum enhanced miniature inhibitory postsynaptic potentials in the
Purkinje neuron probably from presynaptic depolarization induced release of GABA in
the Basket cell/ Purkinje cell synapse. Basket cells are known to provide lateral
inhibition through the activation of GABA_A receptors on the postsynaptic surface of
Purkinje cells as a possible mechanism to fine tune motor movements. Therefore,
modulation of the basket cell-Purkinje neuron synapse would be of interest in studying
motor control by the cerebellum. Our model sought to simulate the activation of GABA_A
receptors on the postsynaptic surface of the Purkinje neuron from evoked activity
(assumed an activation frequency of 50Hz from Hausser and Clark 1997) in the basket
cell in the presence of background activity in which glutameric receptors are also activated on the presynaptic basket cell terminal (12Hz from Hausser and Clark 1997). The simulations were performed under noisy conditions and the transfer of frequency information in the evoked current will be calculated. The kinetic properties of the GABA receptor activation were altered to simulate the effect of a psychoactive drug, chlorpromazine, that affect rates of GABA receptor desensitization and on/off rates. The information processing of the transmitter signal was compared in the presence and absence of chlorpromazine (kinetic parameters from).

We considered the synapse between the inhibitory neuron (basket/stellate cell) and the Purkinje neuron. In this model GABA release from the presynaptic terminal can occur through two mechanisms: 1. Action potentials arriving down the basket cell axon (50Hz pulses that were Poisson distributed, mean GABA amplitude 1.5 mM of pulses that were white noise distributed (evoked activity)); 2. Release of excitatory transmitter, glutamate, in an axo-axonal synapse on the basket cell resulting in GABA release into the presynaptic synapse onto the Purkinje cell (background activity of 12 Hz, GABA amplitude 1.5mM (presynaptic background)). Simulations were performed using the difference equations described above with GABA in the presence or absence of chlorpromazine. In addition to the binding reactions, the clearance of GABA was approximated by a double exponential decay (2311 and 28 sec⁻¹). Using the method
described above, the power spectra of the input signal (averaged from 25 pulse trains) was calculated and shown in Figure 4A. In the absence of background activity (evoked only), the peak amplitude occurs at 50Hz, while in the presence of background activity, a smaller peak was represented at frequency of 12 Hz. In the presence of background and evoked activities there was a greater representation of the signal at all frequencies in the power spectrum. The response to the input frequencies were measured by plotting the response of the two open states of the receptor and comparing the power spectra of the input and the output spectra in terms of signal to noise ratio with GABA binding in the absence (Figure 4A) and presence of chlorpromazine (Figure 4B). Interestingly, in the presence of the pre-synaptic background activity lower signal to noise ratios are observed at the peak driving frequency of 51 Hz (Figure 4B; SNR = 1.93 ± 0.29 with evoked alone compare with SNR = 1.34 ± 0.21 with evoked plus background). When the kinetic parameters were altered with the presence of chlorpromazine, this resulted in greater signal to noise ratios of the activated state of the receptor compared to the stimulation of the receptor with GABA alone across all frequencies as shown in previous studies using this model (compare Figures 4A and 4B). At the 51 Hz frequency band, the evoked signal in the presence of presynaptic background activity (SNR = 24.2 ± 3.0) reduced the signal to noise ratio compared to evoked signal only (SNR = 39.1 ± 5.0). Computational studies investigating the role of the synaptic background activity in neocortical neurons using two stochastic Ornstein-Uhlenbeck processes describing glutamatergic and
GABAergic synaptic conductances showed that the firing rate can be modulated by background activity. The variance of the simulated gamma-aminobutyric acid (GABA) and AMPA conductances individually set the input/output gain; the mean excitatory and inhibitory conductances set the working point, and the mean inhibitory conductance controlled the input resistance. These findings suggest that background synaptic activity can dynamically modulate the input/output properties of individual neocortical neurons. Computational studies designed to investigate the role of synaptic background activity in morphologically reconstructed neocortical pyramidal showed that background activity can be decomposed into two components: a tonically active conductance and voltage fluctuations, and that responsiveness is enhanced if voltage fluctuations are taken into account. Integrative properties of pyramidal neurons using constrained biophysical simulation models suggested that conductance changes reduce cellular responsiveness. Our simulations can be used to test whether the receptors have intrinsic tuning properties that facilitate such modulation of ionic currents by background activity. At driving frequencies of 50Hz synaptic background activity of 12Hz reduces the signal to noise ratio around the 50Hz spectral band. This suggests that inhibitory inputs into a cell would tend to be underrepresented and that the neuron could become more sensitive to excitatory inputs shifting the excitatory-inhibitory balance in the cell.
6. Conclusions.

The first event in signal transduction at a synapse is the binding of transmitters to receptors. Because of rapidly changing transmitter levels, this binding is unlikely to occur at equilibrium. We describe a mathematical approach that models complex receptor interactions in which the timing and amplitude of transmitter release are noisy. We show that exact solutions for simple bimolecular interactions and receptor transitions can be used to model complex reaction schemes by expressing them in sets of difference equations. Since multiple ligands can bind to ionotropic receptors, the equations were extended to describe binding stoichiometry of up to five ligands. Because the equations predict binding at any time, agonist affinity can be calculated by looking at very early time points.

Because it is applicable to noisy systems, we used the difference method to investigate the information processing capabilities of GABA receptors and predict how pharmacological agents may modify these properties. As previously demonstrated, the response to a single pulse of GABA is prolonged through entry into a desensitized state. The GABA modulator chlorpromazine (primarily affects agonist on and off rates) is predicated to increase receptor signal to noise ratio at all frequencies, whereas pregnenolone sulfate (affects receptor desensitization) completely inhibits information transfer. Initial simulations using a model for heterosynaptic regulation shows that signal
to noise ratios can be decreased in the presence of background presynaptic activity both in the presence and absence of chlorpromazine. These types of simulations provide a platform for investigating the effect of psychoactive drugs on complex responses of transmitter-receptor interactions in noisy cellular environments such as the synapse. Understanding this process of transmitter–receptor interactions may be useful in the development of more specific and highly targeted modes of action.

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Figure 1. Phase space plots of GABA binding to the GABA$_A$ receptor.

An invariant manifold is presented plotting O1, B1 and R with increasing concentrations of initial GABA concentrations (A). Rotation in the B1O1 plane is shown in B.

Figure 2. Phase space plot of the Invariant B2-O2-Df manifold.

Invariant manifold from plotting O2, Df and B2 with increasing concentrations of initial GABA concentrations is shown in A. Rotations in the Df-O2 and B2-O2 planes are shown in B and C respectively.

Figure 3 Multiple ligand binding to a receptor.

A. Levels of bound ligand predicted using the exact solution for up to stoichiometry of 5 (u0 set to 1-5; Kd = 1 nM, time of reaction = 3000 sec) with increasing receptor concentration (1 to 20nM). Using excess initial ligand concentration of 10 mM, there is a linear relationship between the amount bound and the stoichiometry at each receptor (total number of binding sites increase with stoichiometry).

B. At a lower initial ligand concentration (10 nM), there is depletion of free ligand concentrations at high receptor and greater stoichiometry of interaction. The resulting curves are different at increasing ligand:receptor ratios.
Figure 4. Heterosynaptic regulation of GABA transmission in Cerebellar Purkinje cells.

GABA transmission between the basket cell and Purkinje neuron (stimulated with an average evoked frequency of 50Hz) was investigated in the presence (triangles with solid line) or absence (squares with dashed line) of stellate cell activity (presynaptic background activity = 12Hz). 25 trains of GABA pulses were simulated (Poisson distributed averages of 50Hz (evoked only) or 50Hz + 12Hz (evoked + presynap)) and the mean (± standard error of mean) power at each frequency band was plotted (A). Binding to the GABA$_A$ receptor was calculated by solving the set of difference equations representing the 3 binding and 4 state transitions. The response of the two open states of the receptor was expressed as the signal to noise ratio with GABA alone (B) or presence of chlorpromazine (C).
Figure 2A
Figure 2B
Figure 2C
Figure 3

A

B
Figure 4

A. Power spectra of GABA release.

B. Signal to Noise ratio of the receptor response with GABA.

C. Signal to Noise ratio of the receptor response to GABA + Chlorpromazine.