Invariant manifolds in a model for the GABA receptor

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Abstract

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

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1 Introduction

Receptor mediated signal transmission in chemical synapses is a fundamental component of neuronal communication. GABAA receptors conduct chloride ions to generate inhibitory post-synaptic currents. Networks of inhibitory neurons are thought to play an important role in modulating neocortical oscillations and hence brain function (Connors & Amital, 1997; Castro-Alamancos & Connors, 1996). Receptor gated ion-channels such as the GABAA receptor can exist in multiple conformational states: bound, unbound, open, closed and desensitized. Since the binding of the transmitter to the receptor is the first constraint in the passing of information from one neuron to the next, we were interested in the relationship between the binding event and the subsequent transition through the different states of the receptor. The GABAA reaction scheme was modeled via basic biochemical processes. It is essentially an adaptation of the fundamental Michaelis-Menten mechanism of enzyme-substrate interactions, and is characterized by a system of

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.
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 has 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 over-all 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. In Section 2, 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 first using standard (explicit) algorithms to nu-
merically 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; for further details we refer the interested reader to (Qazi & Trimmer, 1999). In Section 2, we describe stiffness and present examples which demonstrate the power of implicit methods. In section 3, 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 findings.

2 The GABA reaction scheme

GABA, which is \(\gamma\)-aminobutyric acid, is an inhibitory neurotransmitter (Alberts et al., 1994). The synapse is the site at which neuronal signals are transmitted. The predominant mechanism of signal transmission at the synapse is via chemical exchange from the presynaptic cell to the postsynaptic cell (Qazi & Trimmer, 1999).
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 GABA binding receptors. One such example is the GABAA receptor. 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 (Murray, 1990). Schematically, the reaction looks like

$$R + N \xrightleftharpoons[k_{-1}]{k_1} RN \xrightarrow{k_2} P + N,$$

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,

\[ \dot{R} = -k_1RN + k_{-1}C, \quad \dot{E} = -k_1ES + (k_{-1} + k_2)C, \]
\[ \dot{C} = k_1ES - (k_{-1} + k_2)C, \quad \dot{P} = k_2C. \]

The GABA scheme assumes all the reactions are reversible (ie. all arrows in the reactions (1) 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, 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 appropriate changes become apparent after close examination of the schematic diagrams in Table 1. The GABA reaction variables can be found in Table 2.

As Qazi et al. explain, an adequate model of the GABA interaction with the receptor must have at least two binding sites, two open states and two desensitization states (Qazi & Trimmer, 1999; Jones et al., 1998; Shen et al., 2000; Mozrzymas et al., 1999). Transitions from the slow desensitized \( D_s \) to the fast desensitized state \( D_f \) have also been included in subsequent models (Qazi & Trimmer, 1999;
Table 1: Schematic representation of GABA reactions (Jones et al., 1998; Qazi & Trimmer, 1999).

<table>
<thead>
<tr>
<th>Binding Reactions</th>
<th>State Transitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R + N \xrightarrow{k_{off}} B_1 )</td>
<td>( B_1 \xrightarrow{a_1}{\bar{b}_1} O_1 )</td>
</tr>
<tr>
<td>( B_1 + N \xrightarrow{2k_{off}} B_2 )</td>
<td>( B_1 \xrightarrow{d_1}{\bar{r}_1} D_s )</td>
</tr>
<tr>
<td>( D_s + N \xrightarrow{q} D_f )</td>
<td>( B_2 \xrightarrow{a_2}{\bar{b}_2} O_2 )</td>
</tr>
<tr>
<td>( D_s \xrightarrow{p} D_f )</td>
<td>( B_2 \xrightarrow{d_2}{\bar{r}_2} D_f )</td>
</tr>
</tbody>
</table>

Table 2: GABA scheme variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R )</td>
<td>Receptor concentration</td>
</tr>
<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>
<tr>
<td>( O_1 )</td>
<td>First open state concentration</td>
</tr>
<tr>
<td>( O_2 )</td>
<td>Second open state concentration</td>
</tr>
</tbody>
</table>

Jones et al., 1998; Mozrzymas et al., 1999). These are represented in the seven state model shown in Table 1. Qazi et alia assert that the seven state model is the minimum necessary to predict GABA responses (Qazi & Trimmer, 1999). It is a model which has been well established in the literature (Qazi et al., 1998; Jones et al., 1998; Jones & Westbrook, 1996; Shen et al., 2000; Mozrzymas et al., 1999).
1999).

The GABA reaction scheme is modelled by the system of ODE (2) - (13) 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 time-scales than others. We discuss the numerical simulations of this model in Section 4.
Table 4: GABA reaction scheme parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a_1$</td>
<td>1100 sec$^{-1}$</td>
</tr>
<tr>
<td>$b_1$</td>
<td>200 sec$^{-1}$</td>
</tr>
<tr>
<td>$a_2$</td>
<td>142 sec$^{-1}$</td>
</tr>
<tr>
<td>$b_2$</td>
<td>2500 sec$^{-1}$</td>
</tr>
<tr>
<td>$r_1$</td>
<td>0.2 sec$^{-1}$</td>
</tr>
<tr>
<td>$r_2$</td>
<td>25 sec$^{-1}$</td>
</tr>
<tr>
<td>$p$</td>
<td>2 sec$^{-1}$</td>
</tr>
<tr>
<td>$q$</td>
<td>0.01 sec$^{-1}$</td>
</tr>
<tr>
<td>$d_1$</td>
<td>13 sec$^{-1}$</td>
</tr>
<tr>
<td>$d_2$</td>
<td>1250 sec$^{-1}$</td>
</tr>
<tr>
<td>$k_{on}$</td>
<td>$5 \cdot 10^6$ M$^{-1}$·sec$^{-1}$</td>
</tr>
<tr>
<td>$k_{off}$</td>
<td>$131$ M$^{-1}$·sec$^{-1}$</td>
</tr>
</tbody>
</table>

3 Numerical stiffness

We shall begin this section with a sample dynamical system which exhibits stiffness, and seek to convince the audience that merely using higher-order or adaptive step-size 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 4.

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

$$y'(x) = f(x, y(x)), \quad \text{(14)}$$

$$y(a) = \alpha, \quad x \in [a, b].$$

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 (Hairer et al., 1993), the recursion used to advance the solution of (14) from \( x_n \) to \( x_{n+1} \) is

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

where \( y_n \) is the numerical approximation to the exact solution \( y(x_n) \). The function \( \Phi \) is called the 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.

### 3.1 Stiffness - what is it?

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

**Example 1** Consider the IVP

\[
y' = -10000y - e^t + 10000, \quad y(0) = 1, \quad t \in [0, 5].
\]

The exact solution of this problem is given by

\[
y(t) = -\frac{1}{10001}e^t + 1 + \frac{1}{10001}e^{-10000t}.
\]
We see from the expression above that the last exponential term $\frac{1}{10001}e^{-10000t}$ should become negligible in comparison to 1. Indeed, when $t \approx .072$, this term contributes less than $10^{-15}$ 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 step-size 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

In Table 3.1, we compile some results using various standard explicit numerical 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 con-
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>

ducted in MATLAB, on a Linux workstation with a 1.3GHz Athlon processor. The cputimes 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 $\frac{1}{10000} e^{-10000t}$ 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 (as an exponential, $e^{-kt}$ 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 (Lambert, 1991):

**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 $t_n + h$ 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 couple of implicit solvers:

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 3.1.

<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>Backward Euler</td>
<td>2500</td>
<td>.002</td>
<td>.11</td>
<td>.9851898</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 3.1 summarizes the performance of these implicit algorithms. Despite the nonlinear solve, these algorithms outperform those in Table 3.1 by a couple of orders of magnitude both in terms of number of time-steps, and cputime taken.

### 4 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 (Caberlin, 2002; Caberlin et al., 2002). 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 (Caberlin, 2002; Caberlin et al., 2002).

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

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Table 7: Initial conditions for the GABA scheme

\[ R_0 = 1 \times 10^{-6} \text{ M} \]
\[ N_0 \in (0.5, 4096) \times 10^{-6} \text{ M} \]
All other initial variables set to zero.

### 4.1 Stiffness in the GABA reaction scheme

In an attempt to reproduce Figure 4 of (Qazi & Trimmer, 1999), we first ran \texttt{ode45} on the GABA system (2) - (13). We set the initial GABA concentration to \( N_0 = 5 \times 10^{-7} \text{ M} \) and ran the experiment for \( t \) between 0 and 1 second. We found \texttt{ode45} to be unstable. It starts the integration with a step length of \( 2.5 \times 10^{-4} \text{s} \). Neither setting the initial step size to \( 1.0 \times 10^{-4} \text{s} \), nor the maximum step size to \( 1.0 \times 10^{-3} \text{s} \) makes the solution stable. This can be seen in Figure 1 with the aforementioned maximum step size. With a maximum step size of \( 5 \times 10^{-4} \text{s} \), \texttt{ode45} integrates the system well in 2000 steps. With smaller step sizes, the plots are graphically indistinguishable at this resolution. Note the saturation of plus symbols for the explicit integrators in Figure 2, even after the initial transient phase. This is typical of solving stiff systems using explicit methods. With \texttt{ode23s}, we find good convergence with the maximum step length set to 0.01s. This step length is used by \texttt{ode23s} throughout the interval, completing the integration in 100 steps, a twenty-fold improvement over \texttt{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 3 we see the variables $B_1$ and $O_1$ 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 $D_f$ also become dependent, as seen in Figure 4. The
relationship here is given approximately by $D_f \approx 48.4B_2 \approx 2.73O_2$. 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.
4.2 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 (Jones & Westbrook, 1997; Bianchi et al., 2001). Desensitization tends to prolong the inhibitory current and keeps the transmit-
Figure 4: top: Invariant $B_2-O_2-D_f$ manifold. bottom l: Rotation in $D_fO_2$-plane. bottom r: Rotation in $B_2O_2$-plane.

ter 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 (Connors & Amitai, 1997; Staak & Pape, 2001). 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 (9, 12, 16). 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.

5 Acknowledgements

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