A MATHEMATICAL MODEL OF NEUROCHEMICAL MECHANISMS IN A SINGLE GABA NEURON

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree

MASTER OF MATHEMATICAL SCIENCES

in the Graduate School of The Ohio State University

By

Evelyn Rodriguez, B.S.

Graduate Program in Mathematical Sciences

The Ohio State University

2016

Thesis Committee:

Janet Best, Advisor
Joseph Tien
© Copyright by
Evelyn Rodriguez
2016
ABSTRACT

Gamma-amino butyric acid is one of the most important neurotransmitters in the brain. It is the principal inhibitor in the mammalian central nervous system. Alteration of the balance in Gamma-amino butyric acid neurotransmission can contribute to increased or decreased seizure activity and is known to be associated with Major Depression Disorder [98]. Furthermore, proton magnetic resonance spectroscopy studies have shown that depressed patients demonstrate a highly significant (52%) reduction in occipital cortex Gamma-amino butyric acid levels compared with the group of healthy subjects [49]. These consequences of Gamma-amino butyric acid dysfunction indicate the importance of maintaining Gamma-amino butyric acid functionality through homeostatic mechanisms that have been attributed to the delicate balance between synthesis, release and reuptake. Here we develop a mathematical model of the neurochemical mechanism in a single GABA neuron to investigate the effect of substrate inhibition and knockout in fundamental and critical mechanisms of GABA neuron. Model results suggest that the substrate inhibition in Glutaminase and GAD65 knockout reduce significantly the cellular and extracellular Gamma-amino butyric acid concentration, revealing the functional importance of Glutaminase and GAD65 in GABA neuron neurotransmission.
ACKNOWLEDGMENTS

The completion of this project could not have been possible without the participation and assistance of the following: Dr. Best, Dr. Tien, Dr. Golomb, Dr. Overman, Dr. Terman, Dr. Hojjat, Dr. Thompson, The Ohio State University Neuroscience Department and Stanford Medical School Department of Neurology and Neurological Sciences. Their contributions are sincerely appreciated and gratefully acknowledged. However, I would like to express my sincere appreciation to Roman Nitze for his endless support, patience and for everything he has done for me in order to continue at The Ohio State University while I was going through a hard time. Also, I would like to thank my fiancé, for being always with me at the hardest and happiest moment of my life.
VITA

2013 .............................. B.S. in Mathematics,
                      Pontifical Catholic University, Puerto Rico.

2014 - present ...................... M.S. in Mathematics,
                      The Ohio State University, USA.

FIELDS OF STUDY

Major Field: Mathematical Sciences

Specialization: Mathematical Biosciences
# TABLE OF CONTENTS

Abstract .................................................................................................................. ii
Acknowledgments ................................................................................................... iii
Vita ............................................................................................................................. iv
List of Tables ........................................................................................................ vi
List of Figures ......................................................................................................... vii

CHAPTER | PAGE
---|---
1 | Introduction .......................................................... 1
2 | Background ............................................................. 3
   | 2.1 GABA Neuron ......................................................... 3
   | 2.2 Biosynthesis of GABA ............................................. 4
   | 2.3 GABA Concentration ............................................. 6
   | 2.4 GABA Degradation ............................................... 6
   | 2.5 GABA Release and Uptake ..................................... 7
   | 2.6 GABA Receptors ................................................. 9
   | 2.7 Disorders of GABA Neuron Dysfunctions ............... 10
   | 2.8 Pharmacology and GABA metabolizing enzymes ....... 11
      | 2.8.1 GAD inhibition ................................................ 11
   | 2.9 Transgenic Animals and GABA Metabolizing ......... 12
      | 2.9.1 GAD Knockout Mice ......................................... 12
      | 2.9.2 Vesicular Transporters Knockout Mice ............... 12
      | 2.9.3 Mutations and Vesicular Transporters ............... 12
   | 2.10 MRS for Measuring GABA Metabolism ................. 13
   | 2.11 Parameter Estimation via Nonlinear Regression ...... 13
      | 2.11.1 Nonlinear Regression ..................................... 14
      | 2.11.2 Nonlinear Regression for a Simple Uninhibited Enzyme Substrate Reaction ........................................... 16
      | 2.11.3 Nonlinear Regression for Non-competitive Inhibitor Enzyme Substrate Reactions ............................... 17
2.11.4 Nonlinear Regression for a Competitive Inhibitor Enzyme Substrate Reaction

3 Methodology and numerical simulations

3.1 Mathematical Model
  3.1.1 Reactions Kinetics
  3.1.2 Assumptions

3.2 Parameters Estimation

3.3 Numerical Simulations and Results
  3.3.1 Enzyme Inhibition
  3.3.2 GLS inhibition and GABA concentration
  3.3.3 GAD65 Knockout and GABA concentration
  3.3.4 GAT-1 Knockout and GABA concentration

4 Discussion

5 Conclusion

Bibliography
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Mathematical Model.</td>
<td>23</td>
</tr>
<tr>
<td>3.2 Variables and Abbreviations.</td>
<td>23</td>
</tr>
<tr>
<td>3.3 Parameters (μM, μM/hr, hr⁻¹).</td>
<td>24</td>
</tr>
<tr>
<td>3.3 Parameters (μM, μM/hr, hr⁻¹).</td>
<td>25</td>
</tr>
<tr>
<td>3.4 Concentrations of Substrates (μM).</td>
<td>26</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Diagram based on Rowley and Madsen Neurochemical Scheme.</td>
<td>22</td>
</tr>
<tr>
<td>3.2</td>
<td>Substrate Inhibition of GLS by 6-diazo-5-oxo-l-norleucine is non competitive.</td>
<td>28</td>
</tr>
<tr>
<td>3.3</td>
<td>GLS Inhibition and Cellular GABA Concentration.</td>
<td>29</td>
</tr>
<tr>
<td>3.4</td>
<td>GLS Inhibition and Extracellular GABA Concentration.</td>
<td>29</td>
</tr>
<tr>
<td>3.5</td>
<td>GAD65 knockout mice and Cellular GABA Concentration.</td>
<td>31</td>
</tr>
<tr>
<td>3.6</td>
<td>GAD65 knockout mice and Extracellular GABA Concentration.</td>
<td>31</td>
</tr>
<tr>
<td>3.7</td>
<td>GAD65 knockout mice and Experimental Data.</td>
<td>32</td>
</tr>
<tr>
<td>3.8</td>
<td>GAT-1 knockout mice and Cellular GABA Concentration.</td>
<td>33</td>
</tr>
<tr>
<td>3.9</td>
<td>GAT-1 knockout mice and Extracellular GABA Concentration.</td>
<td>34</td>
</tr>
<tr>
<td>3.10</td>
<td>GAT-1 knockout mice and Experimental Data.</td>
<td>34</td>
</tr>
</tbody>
</table>
The brain is composed of trillions of nerve cells, some of which can produce impulses. These cells are called neurons. When they communicate with one another, circuits are formed which allow you to think, feel, and function properly in response to your surroundings. Specific compounds, known as neurotransmitters, are released by a presynaptic neuron on one side of the synapse and float across the gap to communicate with the postsynaptic neuron. The neurotransmitters bind to specific receptors and either begin another impulse in the postsynaptic neuron or prevent it.

In the mammalian brain, there are two main neurotransmitters: glutamate and Gamma-amino butyric acid [10]. Glutamate is excitatory, which means that when it binds into its corresponding receptor sites, a new impulse is initiated in the postsynaptic neuron. On the other hand, Gamma-amino butyric acid is the principal inhibitor in the mammalian central nervous system [95]. Which means that when it binds into its corresponding receptor sites, the production of a new impulse is prevented. The interaction between GABA and glutamate provides a stable and balanced environment for the brain, under normal conditions. Because of its inhibitory characteristic, widespread presence and utilization, Gamma-amino butyric acid is involved in clinical conditions including psychiatric disorders and seizure [95].
Proton magnetic resonance spectroscopy studies have demonstrated that Gamma-aminobutyric acid could play a key role in major depressive disorder [49]. Scientists have shown that individuals who have major depressive disorder have altered functions of Gamma-aminobutyric acid [98]. This points to the possibility that medications which correct a GABA imbalance could advance the treatment of major depressive disorder. Several current medications for mood disorders correct imbalances in neurotransmitters such as serotonin and dopamine. However, many patients do not benefit from these medications. Generating the idea that some current medications do not help many patients because those drugs don’t affect the Gamma-aminobutyric acid related brain chemistry [108]. Here we develop a mathematical model of the neurochemical mechanisms in a single GABA neuron to investigate the effect of substrate inhibition and knockout in the fundamental and critical homeostatic mechanisms of GABA neuron. The model is described by a system of ordinary differential equations which includes variables related to extracellular concentrations, intracellular concentrations, degradation rates and reaction rates.
CHAPTER 2
BACKGROUND

2.1 GABA Neuron

The brain is the center of the nervous system. In most animals the nervous system is divided into central and peripheral nervous system. The major parts of the nervous system are brain, spinal cord and nerves. Neurons, the basic unit of nervous tissue, send signals to all the cells in the form of electrochemical waves which travel along thin fibers called axons. These signals causes chemicals called neurotransmitters to be released at junctions called synapses. The release of neurotransmitters can be throughout vesicular and non-vesicular compartments [6]. A cell that receives a signal may be excited, inhibited, or otherwise modulated [91].

Neurons secreting gamma-amino butyric acids (GABA) as their primary neurotransmitter are called GABA neurons. GABA is an inhibitory neurotransmitter, in other words, inhibits whichever neurons its binds to. These neurotransmitters are binded with membrane proteins called receptors. Neurotransmitter binds only to a specific receptor, thereby GABA neurotransmitters bind with GABA Receptors [74]. Subclasses of GABA neurons differ in their morphological, biochemical, and functional characteristics, indicating that they play distinct roles in regulating cortical circuitry [11, 38].
2.2 Biosynthesis of GABA

GABA is synthesized from the decarboxylation of glutamate via the action of glutamate decarboxylase (GAD), which is known to regulate the steady state concentration of GABA [10]. Both GABA and GAD are localized to GABA neurons although not exclusively. The synthesis of GABA in the central nervous system takes place primarily in axon terminals, which is then likely to be available for release into the synapse [7].

GAD activity is the rate limiting step in GABA synthesis and seems directly to participate in the synaptic GABA mechanisms. The regulation of GAD activity is linked to the inter-conversion between the cofactor bound and free forms of GAD [8]. GAD exists in two different forms in the brain: GAD65 and GAD67 [121]. The two GAD isoforms show differing kinetic properties and cellular distributions suggesting different functional roles for cellular and vesicular GABA [122, 123]. Within GABA neurons, GAD65 is highly concentrated in nerve terminals as demonstrated by immunocytochemical studies and density gradient fractionation studies [27]. Whereas GAD67 is found throughout the neuron and exists mostly in a cofactor bound active form [7]. GAD65 is associated with synaptic vesicles, whereas GAD67 serves a non-synaptic, intra cellular GABA pool. In addition to its predominant localization in GABA neurons, GAD is also expressed in certain Glutamate neurons. In comparison to neurons, an overwhelming body of evidence indicates that GAD is absent in astrocytes [10].

Alternate pathways of GABA synthesis from polyamine pathways have been suggested. However, GABA synthesis from polyamine pathways is quantitatively minor in the brain even though polyamines appear to play a significant role in the developing brain and retina [101].
Although GABA is known to be produced exclusively in the neuronal compartment, the relative contributions of the two alternative substrates, neuronal glucose or glial glutamine has been controversial. Glutamine was proposed as an important precursor of the GABA pool [84]. Early experiments compared radioactivity of GABA in the intact brain, brain slices, and synaptosomes after treatment by glutamine and glucose [11, 58]. However, the role of glucose as a direct precursor of GABA was difficult to assess in these early experiments because glutamine produced during the glial metabolism of glucose could also contribute to GABA formation. Therefore, it was difficult to distinguish if GABA was produced directly from the neuronal metabolism of glucose or after the formation of glial glutamine [77].

The conclusion of a more recent study claiming that GABA is formed from a glial pool of TCA cycle intermediates derived from the anaplerotic pathway, was based on an erroneous assignment of the NMR signal of GABA as GABA [32,103]. When acetate was used, both glutamine and GABA were significantly labeled as assessed from vivo analysis of brain slices and cultures of cerebral astrocytes and neurons, which has supported suggestions that glutamine is an important precursor for GABA [42, 43]. In contrast, recent in vivo MRS results have shown that the labeling of GABA from acetate is much less than observed in vivo, indicating that the importance of glutamine as a precursor for GABA in the normal brain is less than previously believed. Many studies have also shown that neuronal glucose is quantitatively the major metabolic precursor of GABA in the intact brain [101,102]. In particular, a recent in vivo isotope chase experiment showed that when glutamine was a main precursor available to GABA, the turnover of GABA was extremely slow, further corroborating that glutamine is a minor metabolic pre-cursor of GABA [10].
It has been suggested that GABA synthesis is regulated by product inhibition of GAD65 [114]. In in-vitro experiments, GABA was found to be a competitive inhibitor of GAD. However, intracellular GABA levels, are not sufficiently high enough to regulate GAD. There is no evidence of short term regulation of GAD by second messengers [2,43,52,]. Most experimental evidence has shown that, unless the concentration of GABA is excessively high, GAD activity is not inhibited by GABA [10,95].

### 2.3 GABA Concentration

GABA is ubiquitously distributed in the brain and its inhibitory function is effective with nearly all neurons. The GABA concentration vary quite significantly based on brain regions and specific actions of GABA neurons in each region [7,57,86]. However, the concentration of GABA in astroglia and interstitial fluid remains at a very low level due to highly active GABA metabolism via transaminase activity and active transport [57]. Therefore, a large concentration gradient of endogenous GABA exists between GABA neurons and the neighboring cells under normal physiological conditions. Alteration of GABA concentration is often associated with an altered GABA function including the onset of convulsions as in seizure activity [12,95].

### 2.4 GABA Degradation

GABA is catabolized via Succinic Semi-Aldehyde (SSA) cycle. This metabolic pathway is known as the GABA shunt, which is the major pathway of GABA synthesis and catabolism [95]. When GABA-T is inhibited, there is substantial accumulation of cytosolic and vesicular GABA in the brain [23]. In cell culture studies, GABA-T inhibition has been shown to enhance depolarization release of endogenous GABA.
Similarly, increased electrical stimulation GABA release has also been observed in brain slices treated with GABA-T inhibitors [43].

### 2.5 GABA Release and Uptake

The functional activity in the brain is primarily composed of interplay between excitation and inhibition. In any brain region, the output is based on a complex processing of incoming signals that require both excitatory and inhibitory synapses. In each of these synapses, the neural activity depends on biosynthesis, release, interaction with receptors, and inactivation of the neurotransmitters. The GABA neuron neurotransmission processes are initiated by the release of neurotransmitter GABA and its subsequent interactions with receptors on the postsynaptic neurons and in some cases on presynaptic neurons. GABA is cleared from the synaptic cleft by molecular diffusion and uptake by the presynaptic GABA neuron [35,121].

GABA is released out of the cell via a calcium dependent vesicular mechanism or via calcium independent non-vesicular mechanism [7]. The synaptic action of GABA released from neurons by calcium dependent vesicular release is terminated by uptake mainly into neurons [24]. The uptake carrier derives its energy to accumulate GABA from the transport of two sodium ions and one chloride ion into the cell with each GABA molecule [38]. A carrier with this stoichiometry transports net positive charge into the cell and must be able to run backward, releasing GABA into the extracellular space if intracellular sodium rises high enough or if the membrane potential becomes sufficiently positive. In brain slice and synaptosomal preparations, GABA release can be evoked by depolarization with potassium or glutamate. This can occur even when vesicular release is inhibited by removal of extracellular calcium [121]. Reversal of GABA transporters had been considered to be significant only during pathological
conditions. Recent studies have shown that GABA transporters reverse quite easily and are highly sensitive to changes in GABA concentration gradient and membrane potential [58].

The inactivation of GABA is primarily maintained by a highly efficient GABA transport system. GABA transporters belong to a superfamily of sodium and chlorine dependent neurotransmitter transporters. Among the GABA transporters, GAT-1 is the most abundantly expressed and preferentially located on neurons [77,92]. GAT-3 and GAT-4 are primarily expressed in glia and other non-neuronal cells [92]. It is well established that GABA neuron neurotransmission is based on recycling of neurotransmitter [115]. Most released GABA is taken back into GABA neuron nerve endings, thus enabling its incorporation into GABA vesicles and subsequent re-release [10]. Astroglial GABA transport has also been found to be of functional significance. A correlation between the anticonvulsant activity of GABA transporter inhibitors and their ability to selectively inhibit astroglial GABA uptake has been experimentally demonstrated. In comparison, no such correlation has been demonstrated for neuronal GABA uptake. Recent evidence has clearly shown that inhibitors of astroglial GABA transport can significantly increase the availability of GABA in the synaptic cleft and surrounding areas [50].

The uptake of GABA is sodium dependent. Experimental evidence of the energy requirement of GABA uptake or inhibition has been controversial [10]. Deoxyglucose experiments have shown that inhibition is a metabolically active process. However, fMRI of the inhibited motor cortex of healthy humans showed no measurable changes in the blood oxygen level dependent signal in the motor cortex, indicating minimal metabolic cost associated with inhibition [91]. Glucose metabolism and GABA glutamine cycling have been found to strongly correlate with anesthesia induced changes
in brain activity levels, suggesting significant contribution from GABA neurons and inhibition to cortical energy metabolism. In contrast, no metabolic cost associated with GABA uptake into astrocytes was measurable in cultured mouse cortical astrocytes [69,121]. Since the majority of released GABA is taken up back to GABA neurons, the energy cost associated with neuronal reuptake of GABA appears to be more relevant for assessment of the energy cost of inhibition.

2.6 GABA Receptors

Three general classes of GABA receptor are known: GABA$_A$, GABA$_B$ and GABA$_C$ receptors. The GABA$_A$ receptors are ionotropic receptors and ligand gated ion channels. Upon activation, GABA$_A$ receptors selectively conduct chloride ions into the cell resulting in hyperpolarization of the neuron. This process causes an inhibitory effect of neurotransmission by diminishing the chance of a successful action potential [14,16].

The GABA$_B$ receptors are metabotropic receptors linked via G-protein to potassium channels, therefore they can stimulate the opening of potassium channels which brings the neuron closer to the Nernst Potential, hyperpolarizing the neuron membrane [9,74]. This process prevents voltage-gated sodium channels from opening, action potential from firing and stops neurotransmitter release.

GABA$_C$ receptors are the newly identified member of the GABA receptor family. They are also linked to chloride channels, with distinct physiological and pharmacological properties. In contrast to the fast and transient responses elicited from GABA$_A$ receptors, GABA$_C$ receptors mediate slow and sustained responses which lowers the membrane potential of the neuron and stops the firing of action potentials. GABA$_C$ receptors are expressed in many brain regions, with prominent distributions...
on retinal neurons, suggesting these receptors play important roles in retinal signal processing [123].

2.7 Disorders of GABA Neuron Dysfunctions

A variety of clinical disorders involve GABA metabolism and neurotransmission. It is well established that GABA neuron dysfunction plays a key role in several neurological disorders such as epilepsy and major depressive disorder. Clinical studies have demonstrated that vigabatrin, (GABA-T inhibitor) and 6-diazo-5-oxo-L-norleucine (GLS inhibitor) are effective anticonvulsant drugs in epileptic patients [95,107]. The inhibition of GABA-T by vigabatrin leads to augmented presynaptic availability and release of intracellular GABA and therefore reduced cortical excitability, which has been considered the mechanism by which vigabatrin exerts its anticonvulsant action in epileptic patients [90,94]. Vigabatrin has also been shown to reduce the cerebral metabolic rate for glucose and cerebral blood flow in epileptic patients. The decrease in glucose found to correlate with the increase in total cerebrospinal fluid GABA concentration [77].

In contrast to vigabatrin which has a clear GABA neuron mechanism of action, the possible GABA neuron mechanism of action of valproate has been extensively debated ever since its introduction as an antiepileptic drug. Valporate influences a variety of other parameters which may be relevant to its mechanism of action as an anticonvulsant [48]. Studies investigating the influence of valporate on GABA synthesis have been conflicting, some demonstrated increased GAD levels while others observing decreased GAD levels following long term treatment with valporate [19,48]. Valporate may also inhibit GABA-T. However, the values of reported IC50 concentrations of valporate for the inhibition of GABA-T vary considerably with a
general tendency towards concentrations being above clinically relevant levels [6]. A differential sensitivity of glial and neuronal GABA-T to valporate has also been demonstrated. Neuronal GABA-T was very sensitive to valporate with IC50 values approaching clinically relevant values [48]. Valporate has been found to be able to foment a significant increase in release of intracellular GABA.

Increasing evidence exists to suggest that major psychiatric disorders are associated with perturbations in the metabolism of GABA. Early studies reported that GABA level is abnormally low in the plasma of patients with major depressive disorder [98]. Post mortem studies of spinal fluid GABA concentrations also identified abnormalities in GABA neuron function in depression. Recently, in vivo Magnetic Resonance Spectroscopy (MRS) measurement of cerebral GABA concentrations in patients with major depressive disorders has consistently found reduced GABA levels in both occipital and prefrontal cortices [49,98]. This is consistent with the mood stabilizing and antidepressant effects of some GABA mimetic and anticonvulsant drugs.

2.8 Pharmacology and GABA metabolizing enzymes

2.8.1 GAD inhibition

Glutamic acid decarboxylase is the GABA synthesizing enzyme. Inhibition of GAD, is known to produce convulsions [107]. Since GAD is dependent on pyridoxal phosphate as the co-enzyme, carbonyl trapping agents like derivatives of hydrazine are generally convulsant in nature. Interestingly, amino-oxyacetic acid will act as a convulsant at high doses while at lower doses it is an anticonvulsant. This is best explained by the fact that it inhibits GABA-T more potently than it inhibits GAD.
In this context it should be noted that the convulsant 3-mercaptopropionic acid is a more potent inhibitor of GAD than of GABA-T [105].

2.9 Transgenic Animals and GABA Metabolizing

2.9.1 GAD Knockout Mice

Animals lacking expression of GAD provided insight into the role of these enzymes. GAD knockout mice do not display morphological abnormalities, but may possess altered basal GABA levels and may display spontaneous seizures and increased susceptibility to induced seizures. GAD knockout mice are also more susceptible to increased mortality compared to Wild Type mice [52].

2.9.2 Vesicular Transporters Knockout Mice

Several transgenic animals are available that display alterations in the trafficking, packaging, and release of vesicular stored neurotransmitters. Vesicular GABA Transporter knockout mice exhibit various alterations in synaptic vesicle release, but they do not display anatomical abnormalities. Also, they display spontaneous seizures and increased susceptibility to induced seizures [97].

2.9.3 Mutations and Vesicular Transporters

SNARE complex are proteins which primary role is to mediate the fusion of vesicles with their target membrane bound compartments. There are three major components that form the SNARE complex: SNAP-25, synapto-brevin, and syntaxin. The full knockout of SNAP-25 abolishes exocytotic release and mutants die at birth. SNAP-25 deficiency can lead to seizure activity, as well as pathological changes that
progress with age. Mice completely lacking neuronal synaptobrevin experience impaired neurotransmission, in other words the size and shape of vesicles is impaired while the number and docking are normal [96].

2.10 MRS for Measuring GABA Metabolism

Among many techniques that have been developed to study GABA metabolism, in vivo Magnetic Resonance Spectroscopy (MRS) has the unique ability to monitor GABA metabolic precursors and products in real time. Most MRS methods use spectral editing approaches to suppress the intense creatine methyl proton signal at 3.0ppm and detect the gamma methylene protons of GABA at the same resonance frequency. Various techniques have been developed that include two step editing, double quantum filtering, spectral editing and homonuclear polarization transfer methods. For example, $^{13}C\{^1H\}$ and $^1H\{^{13}C\}$ editing techniques allow one to measure the kinetics of GABA labeling. Most efforts have focused on detecting the labeling of GABA C2, which is converted from glutamate to GABA by GAD. The labeling of the carboxylic GABA C1 has also been used to study GABA metabolism in vivo by $^{13}C\{^1H\}$ MRS [15,67,99].

2.11 Parameter Estimation via Nonlinear Regression

Over the past several years, different enzyme reactions governed by different equations have been identified in the field of biochemistry. Estimation of kinetic parameters of these enzyme reactions is one of the important problems. The nonlinearity in the functional expression for the reaction rate, in terms of substrate concentration and other parameters, makes the parameter estimation problem difficult. Graphical
techniques such as Lineweaver-Burk’s method and the use of Dixon plot to estimate parameters were in vogue for some time. For complex enzyme reactions, such techniques do not furnish reliable results. This has prompted Wilkinson to propose non-linear regression for the estimation of enzyme parameters [116].

2.11.1 Nonlinear Regression

One of the challenges faced in a biochemistry laboratory while studying enzyme kinetics is to decide comprehensive levels of the substrate concentration. For example, it is hard to determine the maximum concentration, minimum concentration, and to know how best to space the intermediate levels and so on. In practice, one may have to plot the data and go through a few iterations before endorsing the data for further processing. After satisfactory acquisition of the data, which invariably carries measurement inaccuracies, enzyme parameters appearing in the governing model can be estimated using the non-linear regression technique.

Consider a functional relationship that expresses the reaction rate $v$ in terms of the independent variable(s) and some parameters. For example, in the case of a simple enzyme-substrate uninhibited reaction it is given by the Michaelis-Menten equation:

$$v = \frac{V_{max}[s]}{K_m + [s]}$$  \hspace{1cm} (2.11.1)

In Equation (2.11.1) above, the substrate concentration $s$ is the independent variable, and $V_{max}$ and $K_m$ are enzyme parameters representing the maximum reaction velocity and the Michaelis-Menten constant respectively. Numerically, $K_m$ equals $s$ when $v = 0.5V_{max}$. In general, there may be more independent variables than $s$ alone, which for convenience, could be put in a vector of $N$ elements say, $\mathbf{x} = [x_1, x_2, \ldots, x_N]^T$. Let the vector $\mathbf{\beta} = [\beta_1, \beta_2, \ldots, \beta_p]^T$ denote a set of $p$ parameters. The dependence of $v$ on $\mathbf{x}$ and $\mathbf{\beta}$ is denoted by $v(\mathbf{x}_m; \mathbf{\beta})$. Let $y_m$ stand for the $m^{th}$
data sample [26]. The subscript \( m \) in all occurrences except in the Michaelis-Menten constant \( K_m \) indicates the \( m^{th} \) observation. Since the measurements are subject to experimental errors, we shall write:

\[
y_m = v(x_m; \beta) + \epsilon_m
\]  

where the measurement error, \( \epsilon_m \sim N(0, \sigma^2_m) \), is assumed to be a normally distributed random variable with a mean zero and a variance of \( \sigma^2_m \). Given a set of measured values of Equation (2.11.2), the parameter estimation problem involves finding an estimate of \( \beta \) that reduces a measure of error, usually the sum of weighted residual squares \( WRSS \), denoted by \( J(\beta) \):

\[
WRSS = J(\beta) = \sum m \{ y_m - v(x_m; \beta) \}^2
\]  

The main objective is to minimize \( J(\beta) \) with respect to \( \beta \). It is hard to find the minimum of \( J(\beta) \) by explicitly differentiating and solving for global minimum. Iterative methods are usually employed [80]. Nonlinear regression is a convenient technique that allows us to estimate the unknown parameters by linearizing Equation (2.11.2) at each iteration starting from an initial guess \( \hat{\beta}_0 \). This is done by first expressing \( v(x_m; \beta) \) with the first two terms in its Taylor’s series expansion around an estimate, initially taken as \( \hat{\beta}_0 \). Then the \( m^{th} \) element in Equation (2.11.2) can be written as:

\[
y_m = v(x_m; \hat{\beta}_0) + \left[ \frac{\partial v(x_m; \beta)}{\partial \beta_1} \frac{\partial v(x_m; \beta)}{\partial \beta_2} \cdots \frac{\partial v(x_m; \beta)}{\partial \beta_p} \right] \bigg|_{\beta=\hat{\beta}_0} \left( \hat{\beta}_1 - \hat{\beta}_0 \right) + \epsilon_m
\]  

where \( \hat{\beta}_1 - \hat{\beta}_0 = \left[ \begin{array}{c} \hat{\beta}_{11} - \hat{\beta}_{10} \\ \hat{\beta}_{21} - \hat{\beta}_{20} \\ \vdots \\ \hat{\beta}_{p1} - \hat{\beta}_{p0} \end{array} \right] \) is assumed to be a small increment in the parameter
vector. This can be done for all observations \( m = 1, \ldots, M \). Now treating the vector \([ y_m - v(x_m; \hat{\beta}_0) ]\) as the residual \( r_0 \) at 0th iteration, we can write:

\[
r_0 \approx \begin{bmatrix}
\frac{\partial v(x_m; \beta)}{\partial \beta_1} & \frac{\partial v(x_m; \beta)}{\partial \beta_2} & \cdots & \frac{\partial v(x_m; \beta)}{\partial \beta_p}
\end{bmatrix}
\bigg|_{\beta = \hat{\beta}_0} (\hat{\beta}_1 - \hat{\beta}_0) + [\epsilon_m]
\]  

(2.11.5)

This process can be iterated by replacing 0 and 1 with \( k \) and \( k + 1 \) respectively. Denoting the matrix involving the derivatives in Equation (2.11.5) at \( k \)th iteration by \( G_k \) we get,

\[
r_k \approx G_k(\hat{\beta}_{k+1} - \hat{\beta}_k) + [\epsilon_m]
\]  

(2.11.6)

The weighted least squares solution for this can be written in terms of generalized inverse of \( G_k \) as:

\[
\hat{\beta}_{k+1} = \hat{\beta}_k + (G_k^T S^{-1} G_k)^{-1} G_k S^{-1} r_k
\]  

(2.11.7)

where

\[
S = (cov[\epsilon_m])
\]  

(2.11.8)

is the covariance matrix of \( [\epsilon_m] \). Equation (2.11.7) helps in estimating the parameters of a given model recursively, but it is in a generalized form \([26, 80, 53]\).

### 2.11.2 Nonlinear Regression for a Simple Uninhibited Enzyme Substrate Reaction

Consider the case of a simple uninhibited enzyme substrate reaction:

\[
v(x; \beta) = \frac{V_{max}[s]}{K_m + [s]}
\]  

(2.11.9)

where \( s \) denotes substrate concentration, \( V_{max} \) the maximum reaction velocity, and \( K_m \) the Michaelis-Menten constant, which equals the substrate concentration at half the maximum rate. Where the variables and parameters are given by:

\[
x = s \quad \text{and} \quad \beta = \begin{bmatrix} K_m \\ V_{max} \end{bmatrix}
\]  

(2.11.10)
The $m^{th}$ element of the residue is given by
\[ r_m = y_m - \frac{V_{max}[s]_m}{K_m + [s]_m} \] (2.11.11)

Elements of the $m^{th}$ row of $G$ matrix is given by:
\[
\begin{bmatrix}
G_{m1} & G_{m2}
\end{bmatrix} = \begin{bmatrix}
-\frac{V_{max}[s]_m}{(K_m + [s]_m)^2} & \frac{[s]_m}{K_m + [s]_m}
\end{bmatrix}
\] (2.11.12)

2.11.3 Nonlinear Regression for Non-competitive Inhibitor Enzyme Substrate Reactions

Let $i$ denote the concentration of inhibitor. The non-competitive reaction is governed by the Michaelis-Menten equation:
\[ v(x; \beta) = \frac{V_{max}[s]}{K_m \left(1 + \frac{i}{k_I}\right) + [s] \left(1 + \frac{i}{k_I}\right)} \] (2.11.13)

where variables and parameters are given by
\[
x = \begin{bmatrix}
s \\
 i
\end{bmatrix} \quad \text{and} \quad \beta = \begin{bmatrix}
 K_m \\
 k_I \\
 V_{max}
\end{bmatrix}
\] (2.11.14)

The $m^{th}$ element of the residue is given by
\[ r_m = y_m - \frac{V_{max}[s]_m}{K_m \left(1 + \frac{i}{k_I}\right) + [s]_m \left(1 + \frac{i}{k_I}\right)} \] (2.11.15)

Elements of the $m^{th}$ row of $G$ is given by Equation (2.11.5).

2.11.4 Nonlinear Regression for a Competitive Inhibitor Enzyme Substrate Reaction

The competitive reaction is governed by the Michaelis-Lenten reaction:
\[ v(x; \beta) = \frac{V_{max}[s]}{K_m \left(1 + \frac{i}{k_I}\right) + [s]} \] (2.11.16)
where variables and parameters are given by

\[ x = \begin{bmatrix} s \\ i \end{bmatrix} \quad \text{and} \quad \beta = \begin{bmatrix} K_m \\ k_I \\ V_{max} \end{bmatrix} \] (2.11.17)

The \( m \)th element of the residue is given by

\[ r_m = y_m = \frac{V_{max}[s]_m}{K_m(1 + \frac{i}{k_I}) + [s]_m} \] (2.11.18)

Elements of the \( m \)th row of \( G \) matrix is given by

\[
\begin{bmatrix}
G_{m1} & G_{m2} & G_{m3}
\end{bmatrix} = 
\begin{bmatrix}
-V_{max}[s]_m k_I(k_I + i) & V_{max}[s]_m K_{m}k_I & [s]_m k_I \\
(K_{m}k_I + K_{m}i + [s]_m k_I)^2 & (K_{m}k_I + K_{m}i + [s]_m k_I)^2 & (K_{m}k_I + K_{m}i + [s]_m k_I)^2
\end{bmatrix}
\] (2.11.19)

If the data points are collected for various inhibitor values, one could vectorize them into a single column \([51,53,90]\). Accordingly, the value for \( i \) in Equation (2.11.16) should be substituted.
3.1 Mathematical Model

3.1.1 Reactions Kinetics

Enzyme kinetics is the study of how biological catalysts increase the reaction rate in biochemical reactions. Without such catalysts, the biological processes necessary for organisms would not proceed at a rate that would sustain life. Reaction rates can be represented mathematically, illustrating how the different chemical species involved in the reaction are affected throughout the reaction.

A simpler way to model reaction rates and enzyme kinetics is implementing the Michaelis-Menten model. The Michaelis-Menten equation is so useful because it involves parameters that can be determined experimentally. The derivation of the model will require algebraic manipulation, but after simplifying the equations we arrive at the Michaelis-Menten kinetics equations in any of the following standard forms:

1. Unidirectional one substrate

\[ v = \frac{V_{\text{max}}[s]}{K_m + [s]} \]  

(3.1.1)
2. Unidirectional two substrates

\[ v = \frac{V_{\text{max}}[s_1][s_2]}{(K_{s_1} + [s_1])(K_{s_2} + [s_2])} \]  

(3.1.2)

Where \( V_{\text{max}} \) and \( k_m \) are the maximum velocity of the reaction and the Michaelis-Menten constant. Using the Michaelis-Menten equations and taking in consideration the following assumptions, we constructed the system of six differential equations on Table 3.1.

### 3.1.2 Assumptions

For this model we considered irreversible reactions since the diagram on Figure 3.1 was developed based on the assumption that all the reactions are unidirectional. In this type of reactions, the reactants convert to products and the products cannot convert back into the reactants.

It is clear that neurons do not depend exclusively on astrocytic glutamine for the replenishment of glutamate. In addition, neurons may synthesize glutamate from TCA cycle intermediates like SSA [95]. Alternatively, SSA can degrade and contribute to the activation of other receptors, but this process was not considered in this model.

After GABA is synthesized it is packaged into vesicles by the Vesicular Gamma-amino butyric acid Transporter (vGAT) [23]. We take the \( K_m \) of the transporter in the literature range and choose the \( V_{\text{max}} \) so that the concentration of cytosolic GABA is in the range \(.1506\mu M - 11.2\mu M\) under normal circumstances. We assume that the majority of the cellular GABA is in the vesicular compartment. The vesicles take up a significant portion of the volume terminal [67]. For simplicity, we are assuming that the cytosol is well mixed and the vesicular compartment is the same size as the non-vesicular compartment since we are not investigating vesicle creation or movement toward the synaptic cleft, where vesicle volume and fusion play a significant role.
Vesicular GABA is put into the synaptic cleft where it becomes extracellular GABA. We represent this process by the term \( fire(t)[vGABA] \), where \( fire(t) \) is a function of time using as reference [13].

For this model we considered three fates for extracellular GABA. It is reuptaked into the cytosol by GAT-1, it is catabolized or it is removed from the system [95]. Cytosolic GABA is catabolized by Succinic Semi-Aldehyde dehydrogenase (SSA) to other particles, which are exported from the neuron and contribute to other metabolic cycles. In this model we are not investigating the details of catabolism, we are only investigating the effect of substrate inhibition and knockout in fundamental and critical mechanisms of GABA neuron. In our model the term \( k_{cGABA}[cGABA] \) represents the catabolism of cellular GABA. After some experimentation we choose \( k_{cGABA}[cGABA] = 3.65/hr \) because it gave good fits to the experimental data.

In our model the extracellular space is a single compartment. For simplicity, we are ignoring diffusion gradients on the extracellular space. The term \( k_{eGABA}[eGABA] \), represents removal of eGABA through the extracellular space. After some experimentation we choose \( k_{eGABA}[eGABA] = 100/hr \) because it gave good fits to the experimental data.

21
Figure 3.1: Diagram based on Rowley and Madsen Neurochemical Scheme.

The figure shows the reactions in the model. The boxes indicate substrates. Ellipses indicate enzymes, transporters and auto-receptors.
Table 3.1: Mathematical Model.

\[
\begin{align*}
\frac{d[eGln]}{dt} &= -V_{SAT}(eGln) - k_{\text{catab}}^{eGln}[eGln] \\
\frac{d[eGln]}{dt} &= V_{SAT}(eGln) - V_{GLS}(eGln) - k_{\text{catab}}^{eGln}[eGln] \\
\frac{d[Glu]}{dt} &= V_{GLS}(eGln) - V_{GAD}(Glu) - k_{\text{catab}}^{Glu}[Glu] \\
\frac{d[cGABA]}{dt} &= V_{GAD}(Glu) + V_{GAT-1}(eGABA) - V_{GAT}(cGABA, vGABA) - k_{\text{catab}}^{cGABA}[cGABA] \\
\frac{d[vGABA]}{dt} &= V_{GAT}(cGABA, vGABA) - fire(t) [vGABA] \\
\frac{d[eGABA]}{dt} &= fire(t) [vGABA] - V_{catab}(eGABA) - V_{GAT-1}(eGABA) - k_{\text{rem}}^{eGABA}[eGABA]
\end{align*}
\]

Table 3.2: Variables and Abbreviations.

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosolic Glutamine</td>
<td>cGln</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Glu</td>
</tr>
<tr>
<td>Cytosolic Gamma-amino butyric acid</td>
<td>cGABA</td>
</tr>
<tr>
<td>Vesicular Gamma-amino butyric acid</td>
<td>vGABA</td>
</tr>
<tr>
<td>Extracellular Gamma-amino butyric acid</td>
<td>eGABA</td>
</tr>
<tr>
<td>Extracellular Glutamine</td>
<td>eGln</td>
</tr>
<tr>
<td>System A Transporter</td>
<td>SAT</td>
</tr>
<tr>
<td>Glutaminase</td>
<td>GLS</td>
</tr>
<tr>
<td>Glutamic Acid Decarboxylase</td>
<td>GAD65</td>
</tr>
<tr>
<td>Vesicular Gamma-amino butyric acid Transporter</td>
<td>vGAT</td>
</tr>
<tr>
<td>Gamma-amino butyric acid Reuptake Transporter</td>
<td>GAT-1</td>
</tr>
</tbody>
</table>
The mathematical model consists of six differential equations for the variables listed in Table 3.2. Reaction velocities or transport velocities begin with a capital \( V \) followed by the name of the enzyme or transporter as a subscript. Our goal is to study the effect of Glutaminase inhibition, GAD65 and GAT-1 knockout on the extracellular and cellular concentration of Gamma-amino butyric acid.

### 3.2 Parameters Estimation

The parameters of the system are given in Table 3.3. Some parameters are based on different sources and others were estimated using experimental data and the method described in section 2.11.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_{SAT} )</td>
<td>System A Transporter</td>
<td></td>
</tr>
<tr>
<td>( K_m )</td>
<td>40</td>
<td>Estimated</td>
</tr>
<tr>
<td>( V_{\text{max}} )</td>
<td>200</td>
<td>Estimated</td>
</tr>
<tr>
<td>( V_{GLS} )</td>
<td>Glutaminase</td>
<td></td>
</tr>
<tr>
<td>( K_m )</td>
<td>64</td>
<td>[79,3,4]</td>
</tr>
<tr>
<td>( V_{\text{max}} )</td>
<td>420</td>
<td>[28,29,33,106]</td>
</tr>
<tr>
<td>( V_{GAD} )</td>
<td>Glutamic Acid Decarboxylase</td>
<td></td>
</tr>
<tr>
<td>( K_m )</td>
<td>40</td>
<td>Model fit</td>
</tr>
<tr>
<td>( V_{\text{max}} )</td>
<td>64</td>
<td>Model fit</td>
</tr>
</tbody>
</table>

Table 3.3: Parameters (\( \mu M, \mu M/hr, hr^{-1} \)).

24 continued
Table 3.3 continued
Parameters (μM, μM/hr, hr⁻¹)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model Values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{GAT-1}$</td>
<td>Gamma-amino butyric acid Reuptake Transporter</td>
<td></td>
</tr>
<tr>
<td>$K_m$</td>
<td>50</td>
<td>[72,35,94,25,73]</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>800</td>
<td>[25,73,110]</td>
</tr>
<tr>
<td>$V_{eGAT}$</td>
<td>Vesicular Gamma-amino butyric acid Transporter</td>
<td></td>
</tr>
<tr>
<td>$K_{eGABA}$</td>
<td>40</td>
<td>Estimated</td>
</tr>
<tr>
<td>$K_{eGABA}$</td>
<td>10</td>
<td>Estimated</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>2400</td>
<td>Estimated</td>
</tr>
<tr>
<td>$V_{catab}$</td>
<td>Catabolic reaction rates</td>
<td></td>
</tr>
<tr>
<td>$K_m$</td>
<td>150</td>
<td>Estimated</td>
</tr>
<tr>
<td>$V_{max}$</td>
<td>300</td>
<td>Estimated</td>
</tr>
<tr>
<td>Catabolism rates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{catab}$</td>
<td>2.50</td>
<td>[56,18]</td>
</tr>
<tr>
<td>$k_{cGln}$</td>
<td>3</td>
<td>[17, 36]</td>
</tr>
<tr>
<td>$k_{Glu}$</td>
<td>6.50</td>
<td>[104,45]</td>
</tr>
<tr>
<td>$k_{eGABA}$</td>
<td>3.65</td>
<td>Model Fit</td>
</tr>
<tr>
<td>$k_{rem}$</td>
<td>100</td>
<td>Model fit</td>
</tr>
</tbody>
</table>
3.3 Numerical Simulations and Results

All simulations were carried out with MAPLESOFT and MATLAB-R2014b over the period of 5 hours. Three methods were used to confirm the solution of the equation: ode15s, ode45 and Runge-Kutta method.

3.3.1 Enzyme Inhibition

There are two main types of enzymes inhibition: competitive and non-competitive. A competitive inhibitor is a molecule other than the substrate that is able to bind to the active site of the enzyme. The enzyme is thus rendered inactive and unable to carry out the reaction. In the presence of competitive inhibitor, the maximum velocity of the reaction does not change, but the rate constants do. This means that the reaction will still approach the same $V_{max}$, but at a given substrate concentration the reaction velocity will be less than when the inhibitor is not present. A non-competitive inhibitor binds to an area that is not the active site of the enzyme, however, its binding causes a conformational change in the enzyme which makes it inactive and unable to bind substrate. In the presence of a non-competitive inhibitor
the rate constant remains the same, but the maximum velocity of the reaction will be lower.

We shall assume that GLS inhibition is non-competitive so we obtain the following expression for the reaction velocity at steady state,

\[ v = \frac{V_{\text{max}}}{1 + \frac{[I]}{K_i} \cdot \frac{[s]}{K_m + [s]}} \]  

(3.3.1)

### 3.3.2 GLS inhibition and GABA concentration

Glutaminase (GLS) converts glutamine into glutamate, eventually glutamate is then converted by Glutamic Acid Decarboxylase (GAD) into Gamma-amino butyric acid (GABA). GLS can be inhibited by CB-839 and 6-diazo-5-oxo-l-norleucine [95]. Here we focus on substrate inhibition of GLS by 6-diazo-5-oxo-l-norleucine. Figure 3.2 shows that the substrate inhibition of GLS by 6-diazo-5-oxo-l-norleucine is non-competitive and we have chosen our kinetic parameters to match the shape of that curve. The non-competitive substrate inhibition of GLS by 6-diazo-5-oxo-l-norleucine implies that GLS can not perform its function of converting glutamine into glutamate, reducing cellular GABA concentration, vesicular stores of GABA and eventually the extracellular GABA concentration. Figure 3.3 - 3.4 show that when we apply GLS inhibition in our model we see changes in half-life of cellular and extracellular GABA concentration. The half-life of cellular GABA declines from 23.55 minutes to 12.27 minutes when GLS is inhibited and the half-life of extracellular GABA declines from 58.66 minutes to 47.77 minutes when GLS is inhibited.

Over the last 20 years, scientists have conducted numerous experiments with glutamate, glutamine and GABA concentration. We focus here on the experiment reported in [124] that examined the glutamine-glutamate-GABA cycle in the lateral
septum (LS) of post-partum female mice. They found that when levels of glutamine or glutamate were elevated or decreased by \textit{in-vivo} modifications in important regulatory mechanisms of post-partum female mice, a significant change in GABA concentration was reported, establishing a positive correlation between glutamine-glutamate-GABA and their respective enzymes. The results in [124] presents a useful model system for understanding how glutamine, glutamate and GABA are linked despite large signaling changes.

Figure 3.2: Substrate Inhibition of GLS by 6-diazo-5-oxo-l-norleucine is non competitive. The two curves plot the velocity of the GLS reaction as a function of cGln for normal Michaelis-Menten for no inhibition and non-competitive substrate inhibition ($K_i = 116\mu M$). In each case $K_m = 64\mu M$. 
Figure 3.3: GLS Inhibition and Cellular GABA Concentration.

The curves plot the time course of cellular GABA concentration in the model before and after inhibition of GLS by 6-diazo-5-oxo-l-norleucine. The half-life of cGABA for WT mice is 23.55 minutes and the half-life of cGABA for inhibited mice is 12.27 minutes.

Figure 3.4: GLS Inhibition and Extracellular GABA Concentration.

The curves plot the time course of extracellular GABA concentration in the model before and after inhibition of GLS by 6-diazo-5-oxo-l-norleucine. The half-life of eGABA for WT mice is 58.66 minutes and the half-life of eGABA for inhibited mice is 47.77 minutes.
3.3.3 GAD65 Knockout and GABA concentration

Glutamine acid Decarboxylase 65 (GAD65) converts glutamate into Gamma-amino butyric acid (GABA). Given the dynamic nature of neurons and the importance of GABA neurotransmitter, it is not surprising that GAD65 knockout will affect GABA concentration and its half-life. Neuroscientist have conducted numerous experiments with GAD65 reporting that GAD65 knockout mice have an increased susceptibility to seizures and a decreased extracellular GABA concentration [52,86,114]. Here we experiment with our model the cases presented in [114] to compare the behavior of cellular and extracellular GABA concentration in wild type mice (WT) and GAD65 knockout mice (KO). To represent the GAD65 knockout mice we set the $V_{max}$ of $V_{GAD}$ to zero. When we modeled GAD65 knockout mice we observed a significant reduction in the half-life of cellular and extracellular GABA concentration compared with the GLS inhibition. This suggest that GAD65 plays a crucial and direct role in GABA synthesis compared to GLS. Figure 3.5 plot the time course curve of cellular GABA concentration before and after GAD65 knockout. As we can observe, the half-life of cGABA for WT mice is 23.55 minutes and the half-life for cGABA KO mice is 1.02 minutes. On the other hand, Figure 3.6 plot the time course curve of extracellular GABA concentration before and after GAD65 knockout. As we can observe, the half-life of eGABA for WT mice is 58.66 minutes and the half-life for eGABA KO mice is 38.90 minutes. Notice that cellular GABA concentration presents a significant decay compared to extracellular GABA concentration, this can be attributed to the fact that GAD65 convert glutamate directly into cellular Gamma-amino butyric acid. If we compare GAD65 knockout results with GLS inhibition results, we conclude that our model is more sensitive to GAD65 knockout than GLS inhibition. This suggest that knockout of GAD65 has major impact on cellular GABA synthesis than GLS.
Figure 3.5: GAD65 knockout mice and Cellular GABA Concentration.

The curves plot the time course of cellular GABA concentration in the model before (WT) and after (KO) knockout of GAD65. The half-life of cGABA for WT mice is 23.55 minutes and the half-life for cGABA KO mice is 1.02 minutes.

Figure 3.6: GAD65 knockout mice and Extracellular GABA Concentration.

The curves plot the time course of extracellular GABA concentration in the model before (WT) and after (KO) knockout of GAD65. The half-life of eGABA for WT mice is 58.66 minutes and the half-life for eGABA KO mice is 38.90 minutes.
On Figure 3.7, we compared our model results with experimental data provided by Stanford University Department of Neurology and Neurological Sciences. In general, we can not expect the model and experimental results to correspond exactly because of oversimplification in our model, measurement inaccuracies and mice conditions in laboratories.

![Image of Figure 3.7: GAD65 knockout mice and Experimental Data.]

3.3.4 GAT-1 Knockout and GABA concentration

A large number of studies have examined the pharmacological and behavioral characteristics of mice that have GAT-1 knocked out. Such knockouts are of particular interest because they represent what one could expect with high doses of particles that block the GATs functionality. In our model, we found that the steady state of vesicular GABA and cellular GABA decline when GATs are blocked. Also, we found that as more GATs are blocked, the steady state of extracellular GABA concentration increases. In our model, we made the GATs knockout by simply setting the \( V_{\text{max}} \) of \( V_{GAT-1} \) equal to zero. We note that in our model, extracellular GABA already rises substantially when only 50% of the GATs are active. On the other hand, vesicular
GABA remains almost normal when only 50% of the GATs are active, suggesting that vesicular GABA concentration decays slowly when GATs are inhibited.

In our model, we find that the half-life of cellular GABA concentration for GATs KO mice is 23.55 minutes and the half-life of cellular GABA concentration for GATs KO is 6.57 minutes. Also, we find that the half-life of extracellular GABA concentration for GATs KO mice is 1.02 hours and the half-life of extracellular GABA concentration for GATs WT mice is 58.66 minutes. When we compares our results for GATs KO mice with experimental data provided by The Ohio State University Neuroscience Department, we observed variation between the data. Once again, we can not expect the model and experimental results to correspond exactly because of oversimplification in our model and conditions of mice in laboratories.

Figure 3.8: GAT-1 knockout mice and Cellular GABA Concentration.
Figure 3.9: GAT-1 knockout mice and Extracellular GABA Concentration.

Figure 3.10: GAT-1 knockout mice and Experimental Data.
We have constructed a basic model for neurochemical mechanism in a single GABA neuron that incorporates the basic known reactions. This model is able to reproduce known properties of GABA metabolism such as the sensitivity of extracellular and cellular GABA concentration to GATs knockout, GAD65 knockout and GLS inhibition. The ultimate goal of our work is not only to summarize or represent the biology that is already known, but to provide a base for further experimentations. Our main focus in this project is to help understand the homeostatic mechanisms involved in GABA synthesis, release and reuptake.

We chose parameter values for the model to be within the published ranges to satisfy the known values of GABA through its neurochemical mechanisms. But, in some cases we simply fitted reasonable values, creating sensitivity around the analysis and mathematical model when parameters were changed.

We have demonstrated that GAD65 plays an important role in cellular GABA concentration and extracellular GABA concentration. We used the model to explain features of the time course of cellular and extracellular GABA in GATs and GAD65 knockout mice. We showed that the model reproduces the results of Zhao and Gammie, where they found that when levels of glutamine or glutamate were elevated or decreased by in-vivo modifications of important regulatory mechanisms of
post-partum female mice, a significant change in GABA concentration was reported, establishing a positively correlation between glutamine-glutamate-GABA.

Any model includes oversimplifications and measurement inaccuracies. We have not included the details of the use of GABA in other metabolic pathways. The processes by which vesicles are created, move to the synapse, and release their GABA are not included in this model, as well the SSA or TCA cycle. In our model the GATs put released GABA back into the terminal, but we do not include leakage of cytosolic GABA through the GATs into the extracellular space. Understanding the homeostasis of different mechanisms between GABA neurons is fundamental for determining new pharmacological strategies for the GABA neuron dysfunctions. Our future goal is to develop a sophisticated mathematical model to explore the variety of proposed hypotheses and the interaction between a network of GABA neurons. As more quantitative information about GABA synthesis, release and reuptake becomes available, it can be incorporated into the model, and further experiments can be tested.
CHAPTER 5
CONCLUSION

In this project we develop a mathematical model of the neurochemical mechanism in a single GABA neuron to investigate the effect of substrate inhibition in the fundamental and critical mechanisms of GABA neuron. Model results suggest that the substrate inhibition in Glutaminase, Glutamic Acid Decarboxylase 65 knockout and GABA reuptake transporter-1 knockout reduce significantly the cellular concentration of Gamma-amino butyric acid, revealing the functional importance of Glutaminase, Glutamic Acid Decarboxylase 65 and GABA reuptake transporter-1 in GABA neuron neurotransmission. Understanding the effects of homeostatic mechanisms in normal and pathological situations is relevant for the implementation of new pharmacological treatments conducted to neuropsychiatric disorders.
BIBLIOGRAPHY


