A Mathematical Model of Dopamine Neurotransmission

by

David Tello-Bravo

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved May 2011 by the
Graduate Supervisory Committee:
Sharon M. Crook, Co-Chair
Priscilla E. Greenwood, Co-Chair
Steven M. Baer
Edward Castañeda
Carlos Castillo-Chávez

ARIZONA STATE UNIVERSITY
May 2012
ABSTRACT

Dopamine (DA) is a neurotransmitter involved in attention, goal oriented behavior, movement, reward learning, and short term and working memory. For the past four decades, mathematical and computational modeling approaches have been useful in DA research, and although every modeling approach has limitations, a model is an efficient way to generate and explore hypotheses. This work develops a model of DA dynamics in a representative, single DA neuron by integrating previous experimental, theoretical and computational research. The model consists of three compartments: the cytosol, the vesicles, and the extracellular space and forms the basis of a new mathematical paradigm for examining the dynamics of DA synthesis, storage, release and reuptake. The model can be driven by action potentials generated by any model of excitable membrane potential or even from experimentally induced depolarization voltage recordings. Here the model is forced by a previously published model of the excitable membrane of a mesencephalic DA neuron in order to study the biochemical processes involved in extracellular DA production. After demonstrating that the model exhibits realistic dynamics resembling those observed experimentally, the model is used to examine the functional changes in presynaptic mechanisms due to application of cocaine. Sensitivity analysis and numerical studies that focus on various possible mechanisms for the inhibition of DAT by cocaine provide insight for the complex interactions involved in DA dynamics. In particular, comparing numerical results for a mixed inhibition mechanism to those for competitive, non-competitive and uncompetitive inhibition mechanisms reveals many behavioral similarities for these different types of inhibition that depend on inhibition parameters and levels of cocaine. Placing experimental results within this context of mixed inhibition provides a possible explanation for the conflicting views of uptake inhibition mechanisms found in experimental neuroscience literature.
A mi adorada esposa: Luz M. Rodriguez,
por su amor, paciencia y comprensión durante los años que tomo terminar esta
dissertation.

Te Amo Mi Reina!

A mi madre: La Sra. Blanca E. Bravo Echeverria,
por todos los sacrificios que ha hecho en esta vida y su amor incondicional.

A mi padre: El Sr. Wilfredo Tello Espinoza,
por haberme traído a este país y enseñarme a luchar por mis metas.

A mis abuelitos: Don Mario Bravo Malpartida y
Doña Carlota Echeverria de Bravo.
Que desde el cielo me cuidan e iluminan mi camino.

A mi abuelita: Teresa Espinoza, si la vida no me dio la oportunidad de conocerte,
ahora que estás en la gloria del Señor y en mi corazón nos conoceremos mejor.

A mis hermanitos: Sheila L. Tello Bravo y Jose F. Zapata Bravo,
tenemos nuestras diferencias, pero los quiero mucho a ambos.

A Rodrigo B. Carramiñana a.k.a “El Gigo,”
por tomarme bajo sus alas y enseñarme a volar.

and

To all immigrants who come to the USA in the
“pursuit of happiness” and the American dream! Si se puede!
ACKNOWLEDGEMENTS

“The miracle is not that I finished.
The miracle is that I had the courage to start!”
- 2007 Chicago Distance Classic Motto.

How long is too long? Who knows? As I write these lines, I can vividly remember the past 16 years of college. My eyes become watery and tears are starting to flow. Throughout the years I have read several studies regarding the success of Chicago Public Schools Latino student graduates, and based on all of the studies, this moment in my life should not be happening. Were the studies wrong? Am I a statistical error? How did I ever get this far? The solution is simple, I got this far due to the common faith of several people and with the help of college loans.

Since it is clear that I never would have come this far without the support of several people, here I wish to acknowledge them by listing them based on the timeframe in which they came into my life. First and foremost, I would like to thank my parents for everything they have done in this life to make sure that I had every opportunity to succeed. From my time at the University of Illinois at Chicago, I would like to thank: the Carramiñana family, the Baldoceda family, the Figueroa family, Ludwin Galindo, Willie Rodriguez, Jose Perales, Noemi and Lupe Rodriguez, Gladys Hansen-Guerra, Beatriz Jamaica, Christina Gonzales, Calixto Calderon, Jeff Lewis, Paul Fong, Melvin Heard, PRSA, LARES, Ronald E. McNair Program, UIC-SROP, IOWA-SROP in particular Phil Kutzko, Eugene Madison and Diana Bryant. From my time at the University of Michigan, I would like to thank: Emilia Huerta-Sanchez, Demosthenous Demosthenis, Geraldine Franco, Leonel Rios-Reyes, Dennis Crespo, Rosa Roman, Ilesanmi Adeboyé, Trachette Jackson, Karen Smith, Alejandro Uribe, Robert Megginson, Peter Scott and Dean Earl Lewis. From my time at ASU, I would like to thank: Arlene
Evangelista, Daniel Romero, Alicia Urdapillete, Luis Gordillo, Cristi Guevara, Roxana Lopez-Cruz, Marco Herrera-Valdez, WAESO, and the School of Mathematical and Statistical Sciences. The following people deserve truly special recognition: Luz M. Rodriguez (my wife), Blanca Bravo (my mom), Wilfredo Tello (my dad), Rodrigo Carramiñana (my mentor), Sharon Lima (my sister from another mother) y familia, Shaojie Chang (the best officemate ever), Eddie Castañeda (my unofficial neuroscience advisor), Agustin Hernandez, William Y. Velez, Randy Eubank and Dennis Crespo-Matos.

A heart felt thank you goes out to my advisor Sharon Crook. As an old Peruvian saying states “si te joden es porque te quieren, y quieren tu porvernir.” I would not be writing these lines if you had not have pushed me to meet my limits. Thanks Profe! Also I would like to thank all of the members of my committee: Priscilla Greenwood, Steve Baer, Edward Castañeda and Carlos Castillo-Chavez.

I have found a truly remarkable proof that shows that a C+ student with ADHD, who is a Chicago public school graduate, almost withdrew from college, and took 6.5 years to get a BS in mathematics is capable of getting a Ph.D., but the margin is too small to write it! :=)

P.S.: On July 4, 2011 while making the final corrections of my dissertation, Vicia Buchanan, one of the best human beings I ever met passed away. Vicia was there for me since my arrival to Chicago in 1993. She was like a grandmother to me, always caring, encouraging, and loving. Her favorite Cuban words to me were “to sabes que te quiero, pero deja de comer mierda y resuelve. Usted es macho y tiene que saber como resolver.” RIP Vicia, you will always live in my heart vieja. :=)
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF TABLES</th>
<th>vii</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>1</td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2 MATHEMATICAL BACKGROUND: NEURAL MODELS</td>
<td>7</td>
</tr>
<tr>
<td>2.1 Conductance-based models</td>
<td>7</td>
</tr>
<tr>
<td>Channel kinetics</td>
<td>7</td>
</tr>
<tr>
<td>Calcium concentration</td>
<td>10</td>
</tr>
<tr>
<td>2.2 Multi-compartmental models</td>
<td>11</td>
</tr>
<tr>
<td>2.3 Dopamine neuron models</td>
<td>12</td>
</tr>
<tr>
<td>Model equations</td>
<td>14</td>
</tr>
<tr>
<td>3 MATHEMATICAL BACKGROUND: MODELS OF NEUROTRANSMISSION</td>
<td>21</td>
</tr>
<tr>
<td>3.1 Functional organization models</td>
<td>21</td>
</tr>
<tr>
<td>3.2 Models of dopamine functional organization</td>
<td>22</td>
</tr>
<tr>
<td>Dopamine synthesis models</td>
<td>23</td>
</tr>
<tr>
<td>Dopamine vesicular-release models</td>
<td>23</td>
</tr>
<tr>
<td>Dopamine overflow models</td>
<td>24</td>
</tr>
<tr>
<td>Variations of the Wightman neurochemical model</td>
<td>26</td>
</tr>
<tr>
<td>Dopamine feedback control models</td>
<td>27</td>
</tr>
<tr>
<td>Dopaminergic synapse models</td>
<td>27</td>
</tr>
<tr>
<td>3.3 Models of dopamine neurotransmission</td>
<td>30</td>
</tr>
<tr>
<td>3.4 Overview</td>
<td>32</td>
</tr>
<tr>
<td>4 MODELING THE TURNOVER PROCESS</td>
<td>33</td>
</tr>
<tr>
<td>4.1 Modeling extracellular DA concentration in the striatum</td>
<td>34</td>
</tr>
<tr>
<td>$J_{rel}$: Calcium-dependent DA release flux</td>
<td>34</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>$J_{DAT}$: Unidirectional flux of DA</td>
<td>37</td>
</tr>
<tr>
<td>$J_{eda}^o$: Outward extracellular flux rate</td>
<td>39</td>
</tr>
<tr>
<td>Establishing parameters</td>
<td>39</td>
</tr>
<tr>
<td>Model results and validation</td>
<td>41</td>
</tr>
<tr>
<td>4.2 Modeling intracellular DA concentration in the striatum</td>
<td>46</td>
</tr>
<tr>
<td>$J_{syn}$: Synthesized DA flux</td>
<td>49</td>
</tr>
<tr>
<td>$J_{VMAT}$: Storage of DA into the vesicular pool</td>
<td>51</td>
</tr>
<tr>
<td>$n_{RRP}$: Average number of readily releasable vesicles</td>
<td>52</td>
</tr>
<tr>
<td>$J_{ida}^o$: Outward intracellular flux</td>
<td>53</td>
</tr>
<tr>
<td>4.3 A model of DA neurotransmission</td>
<td>54</td>
</tr>
<tr>
<td>Model assumptions</td>
<td>55</td>
</tr>
<tr>
<td>Establishing parameters</td>
<td>56</td>
</tr>
<tr>
<td>Model validation</td>
<td>57</td>
</tr>
<tr>
<td>Basal concentrations and fluxes</td>
<td>60</td>
</tr>
<tr>
<td>4.4 Overview</td>
<td>61</td>
</tr>
<tr>
<td>5 MECHANISMS OF INHIBITION OF THE DOPAMINE UPTAKE CARRIER</td>
<td>62</td>
</tr>
<tr>
<td>5.1 Regulatory mechanisms relevant to the functioning of the DA system</td>
<td>63</td>
</tr>
<tr>
<td>5.2 Kinetic analysis of transporters</td>
<td>63</td>
</tr>
<tr>
<td>5.3 Cocaine</td>
<td>69</td>
</tr>
<tr>
<td>Inhibition of the extracellular DA transporter by cocaine</td>
<td>69</td>
</tr>
<tr>
<td>Simulation results for the kinetics of DA-DAT interactions and possible mechanisms of inhibition of DA transport by cocaine</td>
<td>78</td>
</tr>
<tr>
<td>Quantitative comparisons of cocaine affinity</td>
<td>82</td>
</tr>
<tr>
<td>Summary of results</td>
<td>87</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>93</td>
</tr>
<tr>
<td>BIOGRAPHICAL SKETCH</td>
<td>109</td>
</tr>
</tbody>
</table>
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Parameter values for the coupling and spike producing currents of the two-compartment DA model.</td>
<td>18</td>
</tr>
<tr>
<td>2.2 Parameter values for the NMDA current for the two-compartment DA model.</td>
<td>20</td>
</tr>
<tr>
<td>4.1 Parameter values for the extracellular DA compartment described in Section 4.1.</td>
<td>40</td>
</tr>
<tr>
<td>4.2 Parameter values for the intracellular DA compartment described in Section 4.2</td>
<td>58</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td>1.1</td>
<td>Six steps describing the turnover process.</td>
</tr>
<tr>
<td>2.1</td>
<td>Schematic of Kuznetsov et al. (2006) two-compartment DA neuron model.</td>
</tr>
<tr>
<td>2.2</td>
<td>Replica of Kuznetsov et al. (2006) figures 3A and 3C.</td>
</tr>
<tr>
<td>2.3</td>
<td>Replica of Kuznetsov et al. (2006) figures 8A and 8B.</td>
</tr>
<tr>
<td>4.1</td>
<td>Diagram of extracellular DA dynamics.</td>
</tr>
<tr>
<td>4.2</td>
<td>Simulation results for the Kuznetsov et al. (2006) model defined in Chapter 2.</td>
</tr>
<tr>
<td>4.3</td>
<td>Simulation results showing oscillations in the dopaminergic terminal upon arrival of the action potentials shown in the left panel of Figure 4.2 (5.1 Hz frequency)</td>
</tr>
<tr>
<td>4.4</td>
<td>A computational realization of the experiment proposed by Gonon (1988).</td>
</tr>
<tr>
<td>4.5</td>
<td>Simulation results showing oscillations in the dopaminergic terminal upon arrival of the membrane potential described in the right panel of Figure 4.2 (15.9 Hz).</td>
</tr>
<tr>
<td>4.6</td>
<td>Simulation results showing oscillations in the dopaminergic terminal upon arrival of the membrane potential described in Figure 4.2.</td>
</tr>
<tr>
<td>4.7</td>
<td>Model results for the experiment described in Figure 4.4, with $n_{RRP} = 30$.</td>
</tr>
<tr>
<td>4.8</td>
<td>Diagram of intracellular DA dynamics.</td>
</tr>
<tr>
<td>4.9</td>
<td>Diagram of the synthesis pathway for DA.</td>
</tr>
<tr>
<td>4.10</td>
<td>Diagram of the vesicular monoamine transporter (VMAT) dynamics.</td>
</tr>
<tr>
<td>4.11</td>
<td>Simulation results showing oscillations in the dopaminergic terminal upon arrival of the action potentials described in the left panel of Figure 4.2 (5.1 Hz).</td>
</tr>
<tr>
<td>4.12</td>
<td>Simulation results showing oscillations in the dopaminergic terminal upon arrival of the membrane potential described in the right panel of Figure 4.2 (15.9 Hz).</td>
</tr>
<tr>
<td>4.13</td>
<td>Model results for the experiment described in Figure 4.4.</td>
</tr>
</tbody>
</table>
Figure 5.1 Simulation results showing the effects of the intracellular degradation rate $(k_{mao})$ on the percentage change of the concentration of cytosolic and extracellular DA. ................................................. 64

5.2 Simulation results showing the effects of the intracellular degradation rate $(k_{comt})$ on the percentage change of the concentration of cytosolic and extracellular DA. ................................................. 65

5.3 Simulation results showing the effects of the maximal velocity rate of the DAT transporter ($V_{eda,max}$) on the concentration of cytosolic and extracellular DA. ................................................. 66

5.4 Simulation results showing the effects of the concentration of extracellular DA at the half maximal velocity ($K_{EDA}$) on the concentration of cytosolic and extracellular DA. ................................................. 67

5.5 Scheme for DA binding and transport movement. ................................. 68

5.6 Cartoon model of the transport kinetics reaction shown in Figure 5.5 with DAT as the transporter ................................................. 70

5.7 Illustration for the mechanisms that produce mixed inhibition on DAT in the presence of COC. The binding site for DAT with DA and COC illustrates competitive inhibition. A competitive relationship between COC and $Na^+$ along with an allostery relationship between DA and $Na^+$ leads to the expectation of a non-competitive or uncompetitive relationship between DA and COC, suggesting separate binding sites for the binding of DAT (Meiergerd et al., 1994). ................................. 71

5.8 Cartoon model of cocaine actions on the plasma membrane dopamine transporter. ................................................. 72

5.9 Velocity of the uptake of DA as a function of extracellular DA and competitive inhibition by cocaine with $K_{i(COC)} = 58 nM$. ................................................. 74
5.10 Velocity of the uptake of DA as a function of extracellular DA and uncompetitive inhibition by cocaine with $K_{i(EDA-COC)} = 1050\, nM$.

5.11 Velocity of the uptake of DA as a function of extracellular DA and non-competitive inhibition by cocaine with $K_{i(COC)} = 58\, nM$.

5.12 Velocity of the uptake of DA as a function of extracellular DA and non-competitive inhibition by cocaine with $K_{i(COC)} = 270\, nM$.

5.13 Velocity of the uptake of DA as a function of extracellular DA and non-competitive inhibition by cocaine with $K_{i(EDA-COC)} = 1050\, nM$.

5.14 Illustration of the different functional consequences of competitive, uncompetitive, non-competitive and mixed inhibition of the transport of extracellular DA for 1000 $nM$ of COC.

5.15 Mixed inhibition by cocaine (COC) of DA uptake with $K_{i(COC)} = 58\, nM$.

5.16 The effects of cocaine (COC) on $K_{EDA}^{COC}$ and $V_{max,eda}^{COC}$.

5.17 Mixed inhibition by cocaine (COC) of DA uptake with $K_{i(COC)} = 270\, nM$.

5.18 Mixed inhibition by cocaine (COC) of DA uptake with $K_{i(COC)} = 790\, nM$.

5.19 Mixed inhibition by cocaine (COC) of DA uptake with $K_{i(COC)} = 1220\, nM$.

5.20 Mixed inhibition by cocaine (COC) of DA uptake with $K_{i(COC)} = 100000\, nM$.

5.21 Velocity of the uptake of DA as a function of extracellular DA, and mixed inhibition by cocaine with $K_{i(EDA-COC)} = 1050\, nM$ and $K_{i(COC)} = 58\, nM$.

5.22 Percent inhibition as a function of cocaine.
Chapter 1

INTRODUCTION

Cognitive behaviors, physiological functions and emotional reactions are mediated by communication between neurons in the basal ganglia of the brain (Elsworth and Roth, 2004). The basal ganglia is composed of the globus pallidus (external and internal segment), striatum (caudate nucleus and putamen), substantia nigra, and subthalamic nucleus (Wilson, 2004). The substantia nigra along with the ventral tegmental area are found in the midbrain area. Neuronal degeneration in the basal ganglia leads to a diminished concentration of a neurotransmitter called dopamine (DA) and consequently to a deficiency in neural communication. Since its recognition as a neurotransmitter by Carlsson in the 1950s, experimental work has led to theories linking DA to brain diseases such as attention deficit hyperactive disorder, depression, Parkinson’s disease (PD), schizophrenia, and substance abuse (Cooper et al., 2003; Di Chiara, 1997; Elsworth and Roth, 2004; Harsing Jr, 2008; Liu and Graybiel, 1999; Schultz, 2001; Snow and Calne, 1997; Surmeier, 2004; Zaborszky and Vadasz, 2001; Zeiss, 2005).

Dopamine is a catecholamine neurotransmitter abundant in the central nervous system, which is primarily concentrated in the midbrain area (Binder et al., 2001). The distribution of the cell bodies of DA neurons varies dramatically between different mammals (e.g. rodents, primates, and humans) and even more among different vertebrates (Björklund and Dunnett, 2007a). Though most neuroanatomical studies on DA systems have been carried out in rats, other studies have shown that the general anatomical features of the DA system revealed in the rat are also preserved in other mammals (Elsworth and Roth, 2004). The anatomy of a DA neuron consists of a cell body called a soma, from which highly branched extensions called dendrites emerge (Cobb and Abercrombie, 2003; Grace and Bunney, 1983; Roth, 2004). The axon of a DA neuron rises out from a major dendrite (Cobb and Abercrombie, 2003; Grace and
Midbrain dopaminergic neurons (MDNs) are involved in attention, goal oriented behavior, motor functions, reward learning, and short-term and working memory (Di Chiara, 1997; Elsworth and Roth, 2004; Seamans, 2007; Zaborszky and Vadasz, 2001). The cell bodies of these DA neurons are located in the retrorubral area, substantia nigra (SN) and ventral tegmental area (VTA). MDN projecting pathways that originate in the substantia nigra-ventral tegmental area are classified into three distinct systems that target the neostriatum, limbic cortex, and other limbic areas. These are typically referred to as the nigrostriatal (also known as mesostriatal), mesocortical and mesolimbic pathways, respectively (Björklund and Dunnett, 2007a). MDNs release neurotransmitter from their somas (Cobb and Abercrombie, 2003), dendrites (Cobb and Abercrombie, 2003; Glowinski et al., 1979), varicosities (Groves et al., 1998; Seamans, 2007), and axonal terminals (terminal boutons) (Cobb and Abercrombie, 2003; Crocker, 1994). Release may be synaptic, where released DA binds to a post-synaptic receptor on the target cell across the synapse (Agnati et al., 1995), or it may be non-synaptic where released DA diffuses in the extracellular space (ECS), eventually binding with distal receptors (Binder et al., 2001; Groves et al., 1998; Schmitz et al., 2003; Seamans, 2007). The cellular mechanisms for DA release are functionally the same at all release sites and the biochemical cascades involved in this process create the turnover process (Cooper et al., 2003). See Figure 1.1.

Dopamine background concentration varies across the target regions of the DA pathways previously mentioned. Experimental evidence suggests that DA release occurs in the two forms described below (Dreher and Burnod, 2002; Grace, 1991; Robinson
Figure 1.1: **Six steps describing the turnover process:**

1. **Synthesis** is the production of the neurotransmitter by the conversion of the precursor tyrosine (TYR) to di-hydroxyphenylalanine (L-DOPA) via the enzyme tyrosine hydroxylase (TH). L-DOPA is subsequently converted to DA in the presence of the enzyme DOPA decarboxylase (DDC).

2. Newly synthesized DA is located in the cytosol awaiting storage into vesicles via the vesicular monoamine DA transporter (VMAT) protein located on the vesicular membrane.

3. DA remains sequestered in vesicles and is protected from degradative enzymes until it is released via calcium mediated voltage sensitive exocytosis.

4. **Receptor activation** by extracellular DA involves stimulation of postsynaptic receptors during neurotransmission (in the axonal terminal region) and autoreceptors involved in providing feedback to the presynaptic DA neuron (in the somatodendritic region and at the terminal bouton).

5. **DA reuptake** occurs very fast via the DA plasma membrane transporter (DAT) and helps recycle most of the released DA back into the terminal.

6. **Degradative enzymes** deactivate the neurotransmitter intracellularly and extracellularly to halt communication. Adapted from Cooper et al. (2003) and Harsing Jr (2008).
1. **Transient DA release.** DA release in a transient or phasic mode occurs over a short time scale (subseconds to seconds). The amount of DA released in this form depends primarily on the firing mode of MDNs, since evidence suggests that DA release via burst patterns is twenty to thirty times higher than via single spike patterns. See Figure 1 in Grace (1991).

2. **Background DA release.** Long term DA release in a background or tonic mode occurs over a time scale of minutes to hours. This mode controls the background DA concentration level in the ECS, and the amount of DA release is independent of the firing mode of MDNs. As such, background DA release is believed to be affected primarily by functional changes in release or reuptake and may be affected by glutamatergic cortical inputs at DA release sites in the striatum. See Figure 2 in Grace (1991). It is worth noting that an increase in background DA release raises the DA background concentration level. In turn, this triggers DA receptor activation which subsequently, regulates the response of the DA system. On the other hand, decreases in background DA levels may activate homeostatic responses to restore background DA receptor activity to baseline levels. In turn, this increases DA synthesis production and DA release, which restores the background DA level to the original concentration (Grace, 1991; Weiner and Joel, 2002).

In the neuroscience community, it is widely accepted that under normal conditions, the cytosolic pool of DA is mostly composed of newly synthesized DA (Leviel and Guibert, 1987; Parker and Cubeddu, 1985). It is hypothesized that following mid-brain neural lesions, compensatory responses in the remaining neurons lead to homeostatic changes in the dynamics of one or more steps in the turnover process. These
changes could occur due to increased rates of DA synthesis, increased axoplasmic transport, increased vesicular storage, increased vesicular release due to changes in the function of docking proteins, decreased autoreceptor activity, decreased reuptake and recycling, or decreased degradative enzyme activity (Paquette et al., 2009; Snow and Calne, 1997). Unfortunately, there is a fundamental gap in knowledge about the neural adaptations of compensatory mechanisms; hence in this work a mathematical model is constructed to investigate the nature of the dynamic processes involved in turnover and the role that they play in functional changes in neurotransmission at the molecular level under different conditions.

The organization of the dissertation is outlined here. Chapter 2 provides the mathematical background for the modeling studies of excitable cells and their application to dopaminergic neurons. The details of well known mathematical techniques for Hodgkin-Huxley type models and the mechanism for action potential generation in the Wilson-Callaway (Wilson and Callaway, 2000) and Kuznetsov-Kopell-Wilson (Kuznetsov et al., 2006) DA neuron models are also explained. Chapter 3 introduces the background for compartmental models that describe the functional organization of DA dynamics. Models describing the functional and behavioral organization of release sites are briefly discussed. Chapter 4 introduces a single compartment model of extracellular DA concentration dynamics at the nerve terminal driven by simulated membrane potentials from the Kuznetsov-Kopell-Wilson DA neuron model (Kuznetsov et al., 2006), and numerical solutions are computed. The model is extended by incorporating additional compartments to study the effects of DA synthesis, the vesicular monoamine DA transporter (VMAT), and the DA plasma membrane transporter (DAT) behavior. Finally, Chapter 5 presents computational studies based on the variation of parameters in the model that are relevant in the study of cocaine induced dopamine release. We also examine mechanisms for inhibition of DAT by cocaine and provide a context for understanding conflicting experimental results by comparing results for
mixed inhibition to those for competitive, uncompetitive and non-competitive mechanisms.

Quantitative and qualitative approaches are used in analyzing and interpreting model results, and model predictions will facilitate a more comprehensive understanding of the adaptive nature of neurotransmission and the capacity for neurons to compensate for changes due to the introduction of drugs or during neurodegenerative processes. Some of predictions of these modeling studies might be possible to test in a laboratory environment through pharmaceutical approaches targeting specific mechanisms within each step of the turnover process.
Chapter 2
MATHEMATICAL BACKGROUND: NEURAL MODELS

This chapter contains an overview of well known mathematical and computational models for excitable cells and their application to dopaminergic neurons. A large variety of models of a deterministic and stochastic nature (Freeman, 1992; Koch, 1999; Tuckwell and Feng, 2004) have been used to model concepts such as cell activity (Hoppensteadt, 1981; Terman, 2005), synaptic interactions (Mato, 2005), and dendritic morphology (Gerstner and Kistler, 2002). Models of firing activity focusing on reproducing action potentials or spiking behaviors using ion channel dynamics are known as conductance-based models (Nikita and Tsirogiannis, 2007).

2.1 Conductance-based models

Conductance-based models are typically based on the Hodgkin-Huxley formalism for a model of an excitable membrane. For a complete derivation of the Hodgkin-Huxley model see Cronin (1987). These models, for the most part, are based on experimental data and are primarily used to investigate questions related to the interplay between ionic currents and other single cell dynamics (Mishra et al., 2006). They simulate the conductance changes due to channel gating, which yields a set of deterministic or stochastic differential equations aiming to describe the process. Variations among the models are mostly due to the choice of channels and the parameters of the resulting systems of equations.

Channel kinetics

Mathematical models for ion channel kinetics presented in this chapter follow the Hodgkin-Huxley model formalism for excitable membrane (Hodgkin and Huxley, 1952). Previously published studies use experimentally derived data to create kinetic models that capture the essential dynamics of specific ion channels known to exist in dopaminergic neurons; see for example McNaughton and Randall (1997). In general, each ionic
current density $I_{\text{ion}}$ (in $\mu A/cm^2$) is modeled as the product of the channel conductance and the potential difference between the membrane voltage and the ionic equilibrium voltage, that is

$$I_{\text{ion}} = g_{\text{ion}} \cdot (E_{\text{ion}} - V)$$  \hspace{1cm} (2.1)

where $g_{\text{ion}}$ (in $mS/cm^2$) is the conductance for the channel type per unit of surface area, $E_{\text{ion}}$ (in $mV$) is the reversal (Nernst) potential for the corresponding active ion, and $V$ (in $mV$) is the transmembrane potential. In turn, each channel conductance is modeled as the product of the channel’s maximal conductance, $g_{\text{ion}}$, and the fraction of channels that are open

$$g_{\text{ion}} = \bar{g}_{\text{ion}} \cdot m^y \cdot h^z.$$  \hspace{1cm} (2.2)

In equation (2.2), $m$ and $h$ are the activation and inactivation gating variables, respectively, and $y$ and $z$ are small integers that determine the influence of the gating processes on the conductance. In the case of a non-inactivating current, there is no gating variable $h$ and if gating is very fast, the gating variable may sometimes be replaced with its steady-state function, $m_\infty$ or $h_\infty$ as defined in equation (2.5) or (2.7). The gating kinetics of the ionic conductances are governed by equations of the form

$$\frac{dw}{dt} = \alpha_w(V)(1 - w) - \beta_w(V)w.$$  \hspace{1cm} (2.3)

Typically chosen to match experimental data, the voltage-dependent rate function $\alpha_w(V)$ describes the rate by which the channels switch from a closed to open state and $\beta_w(v)$ is the rate for the open to closed transition (Ferreira and Marshall, 1985). An alternative way of writing equation (2.3) is through its steady-state form given by
\[
\frac{dw}{dt} = \frac{w_\infty(V) - w}{\tau_w(V)}
\] (2.4)

where

\[
w_\infty(V) = \frac{\alpha_w(V)}{\alpha_w(V) + \beta_w(V)}
\] (2.5)

is the steady-state activation or inactivation function and

\[
\tau_w(V) = \frac{1}{\alpha_w(V) + \beta_w(V)}
\] (2.6)

determines the time scale of the exponential decay or saturation of the dynamic gating processes. The steady-state activation and inactivation functions are typically given by

\[
w_\infty(V) = \frac{1}{1 + \exp\left[-\frac{(V - V_H)}{V_S}\right]}
\] (2.7)

where \(V_H\) (in mV) is the half activation or inactivation voltage for the gating function and \(V_S\) (in mV) is the activation or inactivation sensitivity. For more details, see Hille (2001) or Keener and Sneyd (2009). Using ionic currents modeled as described above, the current balance equation for the excitable membrane is

\[
C_m \frac{dV}{dt} = \sum_{i_{ion}} I_{i_{ion}} + g_L(E_L - V) + I_{syn} + I_{app},
\] (2.8)

where \(C_m\) (in \(\mu F/cm^2\)) denotes the membrane capacitance, \(g_L(E_L - V)\) (in \(\mu A/cm^2\)) denotes an unknown voltage independent background or leak current, \(I_{syn}\) (in \(\mu A/cm^2\)) is the synaptic current if present, and \(I_{app}\) (in \(\mu A/cm^2\)) mimics the application of injected current during neurophysiological experiments.
Calcium concentration

While the original Hodgkin-Huxley model characterizes voltage-gated ion channels, there are many types of ion channels which are controlled by factors other than membrane potential, including ligand-gated channels, which vary their conductance in response to changes in the intracellular concentration of some other molecule. The most common signaling molecule is calcium, and a widely-used simple model for the changes in intracellular calcium concentration assumes that the concentration increases due to an inward flux of calcium ions through calcium ion channels when calcium currents are activated and that a linear pumping process through the plasma membrane removes calcium ions from the cell (Medvedev et al., 2003). The total calcium in a cell or cellular compartment consists of free calcium and calcium bound to a buffer; hence, the equation that represents the dynamics of the free calcium concentration \([Ca^{2+}]\) (in \(\mu M\)) is

\[
\frac{d}{dt}[Ca^{2+}] = \frac{2\beta}{r} (\alpha I_{Ca} - P_{Ca}[Ca^{2+}]), \tag{2.9}
\]

where \(\beta\) is the ratio of free to buffered calcium and \(r\) (in \(\mu m\)) denotes the radius of the compartment. The parameter \(\alpha\), which converts calcium current into calcium flux, is defined as the inverse of the product of Faraday’s constant \((F = 0.096485 \text{ C/}\mu\text{ mol})\) and the valence of calcium, \(z_{Ca}\), i.e. \(\alpha = 1/(z_{Ca} \cdot F)\). Finally, the parameter \(P_{Ca}\) (in \(\mu m/\text{ms}\)) represents the maximum pump rate surface density (Li et al., 1996; Kuznetsov et al., 2006; Wilson and Callaway, 2000). When modeling an ionic current that is dependent on the calcium concentration, such as \(I_{K(Ca)}\), the conductance is often modeled as an instantaneous function of \([Ca^{2+}]\) using a Hill equation. See for example equation (2.16).
2.2 Multi-compartmental models

Modeling the behavior of a single neuron often requires detailed dynamics for a particular neuron type, since each type of neuron is characterized by distinct electrophysiological and morphological features (Jaeger, 2005; Tretter and Scherer, 2006). As described in Chapter 1, dopaminergic neurons have very complex spatial structures and different regions may contain different ionic channels with different gating kinetics. Conductance-based models of DA neurons follow two basic approaches: (i) ordinary differential equations to model DA neurons as a multi-compartment cell where compartments are coupled electrotonically (Canavier and Landry, 2006; Guzman et al., 2009; Komendantov and Canavier, 2002; Komendantov et al., 2004; Krupa et al., 2008; Kuznetsov et al., 2006; Kuznetsova et al., 2010; Li et al., 1996; Medvedev and Kopell, 2001; Medvedev et al., 2003; Medvedev and Cisternas, 2004; Migliore et al., 2008; Wilson and Callaway, 2000) and (ii) single compartment models (Amini et al., 1999; Canavier, 1999; Canavier et al., 2007; Kuznetsov et al., 2006; Oprisan and Canavier, 2006; Oprisan, 2009; Penney and Britton, 2002; Wilson and Callaway, 2000). For the multi-compartment model approach, a DA neuron is divided into a large number of small pieces or compartments. Within each compartment, the properties of the neuronal membrane are specified. Neighboring compartments are then connected by an axial resistance, resulting in a large system of coupled ordinary differential equations, which specifies the membrane potential at discrete locations along the neuron. The use of a multi-compartment model allows us to characterize how the membrane properties due to cell structure, ion channel distributions, and distributions of synaptic inputs affect stimulus-response behavior under various conditions. Compartments may represent somatic, dendritic, or axonal membrane; they may be passive or excitable and may contain a variety of synaptic inputs. Each compartment is isopotential and spatially uniform in its properties. Nonuniformity in physical properties (diameter changes, electrical properties, etc.) and differences in potential occur between compartments
rather than within them.

2.3 Dopamine neuron models

Conductance-based models of midbrain DA neurons have been used to study the in vitro and in vivo properties involved in the different types of firing modes. These models build primarily on the work of Li et al. (1996), Canavier (1999), Amini et al. (1999), and Wilson and Callaway (2000) and have been used to reproduce experimental results as well as to investigate hypothesis such as that sodium dynamics drive oscillation (Canavier, 1999). The models have also examined the effects of pharmacological agents (Li et al., 1996; Wilson and Callaway, 2000) and explored two types of calcium-dependent firing patterns that DA cells exhibit in vitro (Amini et al., 1999).

Action potentials encode information in their frequency and pattern in neuronal cell bodies, while they serve as propagators of signals in axons. Detailed descriptions of action potential mechanisms of DA neurons are available in Bean (2007), Liss and Roeper (2009), and Shi (2009). Physiologically, MDNs of freely moving rats exhibit three different types of firing modes: regular (clock-like) single spikes, irregular single spikes, and burst firing (Hyland et al., 2002). Although MDNs spontaneously discharge action potentials that last on average 1.5 ms and have a mean firing rate of 3.7 ± 1.5 Hz (Gonon, 1988; Hyland et al., 2002), a significant number of these action potentials occur within a burst (Hyland et al., 2002). Grace and Bunney (1984a) define a burst as a sequence of spikes starting with an interspike interval (ISI) of less than 80 ms and ending with the concurrence of two spikes with an ISI greater than 160 ms. Several studies have further defined a burst as being composed of at least three spikes (two ISIs meeting the criteria) or have defined it on the basis of a single short interval (a pair of spikes) (Hyland et al., 2002). In freely moving rats, most bursts consist of 2.2 ± 0.4 spikes whose ISI is approximately 49.75 ± 9.26 ms, and on average occur every 300 ± 400 ms. Furthermore, the mean intraburst interval is approximately 50 – 66.33
Wilson and Callaway (2000) introduce a multi-compartment model to study a possible dendritic oscillatory mechanism that influences background firing in the SN-VTA area. The model is presented as a coupled oscillator model of the dopaminergic neuron and contains a soma compartment and five small electrically coupled dendritic compartments varying in diameter, and with different natural spiking frequencies (Wilson and Callaway, 2000). The model describes the interaction of voltage and calcium concentration in the dendritic compartments and although it does not provide an accurate representation of some morphological features found in DA neurons, it is capable of qualitatively reproducing most of the effects seen in calcium-imaging experiments such as the gradual buildup of average free calcium in the soma and the overshoot of average calcium in the smaller dendritic compartments (Wilson and Callaway, 2000). The model, capable of reproducing the slow membrane potential oscillations that occurs spontaneously in vitro, suggests that the currents responsible for this oscillation exist in both the soma and the dendrites of DA neurons. Furthermore, the model shows that under resting conditions, the membrane potential in the dendrites and soma compartments oscillates at a frequency similar to the somatic natural frequency. For more details see Wilson and Callaway (2000).

Wilson and Callaway’s (2000) model was extended by Kuznetsov et al. (2006) by adding spike producing sodium, $I_{Na}$, and delayed-rectifier potassium, $I_{K_d}$, currents. They also consider N-Methyl-D-Aspartate (NMDA) and other types of synaptic conductances. In particular, NMDA is included in order to study how DA cell bursting dynamics depends on the activation of dendritic synaptic inputs through NMDA receptors as observed experimentally (Kuznetsov et al., 2006). This new model consists of only two compartments: a single large soma and multiple small identical dendritic compartments treated as a single larger isopotential compartment (Figure 2.1). In the
model, both soma and dendrites contain spike producing sodium ($I_{Na}$) and delayed-rectifier potassium ($I_{K}$) currents where these currents alone were not able to generate repetitive firing. In addition, both compartments contain a voltage-dependent L-type calcium current ($I_{Ca,L}$) that determines the intracellular calcium concentration, a small voltage-dependent potassium current ($I_K$) that prevents large amplitude calcium spikes, a calcium-dependent potassium current ($I_{K(Ca)}$), and a small linear leak current ($I_L$).

In what follows, we will use this DA neuron model to drive our model of DA neurotransmission; thus, we provide further details of the Kuznetsov-Kopell-Wilson model here.

![Figure 2.1: Schematic of Kuznetsov et al. (2006) two-compartment DA neuron model demonstrating the location of various ion currents in the model.](image)

**Model equations**

Each compartment contains a differential equation for the membrane potential and a differential equation for intracellular calcium concentration. The current balance equation for the soma is provided in equation (2.10). The current balance equation for the lumped dendrite is given by equation (2.11), where $I_{NMDA}$ denotes the NMDA cur-
rent. Throughout what follows, subscripts $s$ and $d$ correspond to the soma and dendrite compartments respectively.

\[
\begin{align*}
C \frac{dV_s}{dt} &= I_{app} + I_{K_s}(V_s, n_s) + I_{Na}(V_s, h_s) \\
&+ I_{Ca,L}(V_s) + I_{K}(V_s) + I_{K(Ca)}(V_s, [Ca^{2+}]_s) + I_L(V_s) \\
&+ n_d \cdot g_c \cdot \frac{r_d^2 r_s}{l_s (l_s r_s^2 + l_d r_d^2)} (V_d - V_s) \\
\end{align*}
\]

\[
\begin{align*}
C \frac{dV_d}{dt} &= I_{K_s}(V_d, n_d) + I_{Na}(V_d, h_d) \\
&+ I_{Ca,L}(V_d) + I_{K}(V_d) + I_{K(Ca)}(V_d, [Ca^{2+}]_d) + I_L(V_d) \\
&+ I_{NMDA}(V_d) \\
&+ g_c \cdot \frac{r_s^2 r_d}{l_d (l_d r_d^2 + l_s r_s^2)} (V_s - V_d).
\end{align*}
\]

In equations (2.10) and (2.11) $V_s$ and $V_d$ represent the membrane potentials in the soma and the dendrite respectively, $I_{app}$ is the applied current (as current density, in $\mu A/cm^2$), and $n_d$ denotes the number of small identical dendritic compartments attached to the soma. The intrinsic currents are defined as in equation (2.1). In particular, the spike generating sodium and delayed rectifier potassium conductance are defined as

\[
g_{Na}(V, h) = \overline{g}_{Na} \cdot m_\infty^3(V) \cdot h
\]

\[
g_{K_s}(n) = \overline{g}_{K_s} \cdot n^4
\]

where $\overline{g}_{Na}$ and $\overline{g}_{K_s}$ represent the maximal conductances for the sodium and delayed-rectifier potassium channels respectively, $m_\infty$ is the instantaneous sodium activation steady-state function defined as in equation (2.5), $h$ is the gating variable for sodium inactivation, and $n$ is the gating variable for activation of the delayed rectifier potassium conductance. The activation and inactivation variables for the delayed rectifier potassium conductance and the sodium conductance, respectively, are governed by the
kinetics outlined in equation (2.3). The voltage dependent calcium conductance, \( g_{Ca,L} \), is defined as

\[
g_{Ca,L}(V) = \overline{g}_{Ca} \cdot \left( \frac{\alpha_c(V)}{\alpha_c(V) + \beta_c(V)} \right)^4
\]  

(2.14)

with the gating treated as instantaneous. The gating functions \( \alpha_c(V) \) and \( \beta_c(V) \) are defined as in equation (2.3), and \( \overline{g}_{Ca, L} \) denotes the maximal conductance. The voltage dependent potassium conductance, \( g_K \), is defined by the Boltzman function

\[
g_K(V) = \overline{g}_K \cdot \frac{1}{1 + \exp \left( \frac{-(V-V_{HK})}{V_{SK}} \right)}
\]  

(2.15)

where \( \overline{g}_K \) represents the maximal conductance, \( V_{HK} \) is the half-activation voltage, and \( V_{SK} \) denotes the voltage sensitivity for activation (Kuznetsov et al., 2006; Wilson and Callaway, 2000). The calcium-dependent potassium conductance, \( g_{K(Ca)} \), uses a fourth power Hill equation of calcium concentration to represent the characteristics of a small conductance (SK) channel and is defined as

\[
g_{K(Ca)}([Ca^{2+}]_{i_x}) = \overline{g}_{K(Ca)} \cdot \frac{[Ca^{2+}]_{i_x}^4}{[Ca^{2+}]_{i_x}^4 + K_{Ca}^4}
\]  

(2.16)

where \( \overline{g}_{K(Ca)} \) denotes the maximal conductance, \( K_{Ca} \) (in \( \mu M \)) denotes the half-activation calcium concentration, and the subscript \( x \) denotes \( s \) when the calcium channel is in the soma and \( d \) when it is in the dendrite (Canavier et al., 2007; Komendantov et al., 2004; Kuznetsov et al., 2006; Li et al., 1996; Oprisan, 2009; Wilson and Callaway, 2000).

The voltage dependent NMDA conductance follows the expression presented in Li et al. (1996) given by
\[ g_{NMDA}(V_d) = \overline{g}_{NMDA} \cdot \frac{1}{1 + \frac{[Mg^{2+}]_o}{10} \cdot \exp\left(\frac{-V_d}{12.5}\right)} \] (2.17)

where \( \overline{g}_{NMDA} \) is the maximal conductance and \([Mg^{2+}]_o\) is the external Mg\(^{2+}\) (in mM) concentration. The changes in intracellular calcium concentration in the soma or the dendrite compartment mimic equation (2.9) and increase due to \( I_{Ca,L} \). The coupling for the two compartments is approximated using geometrical and biophysical properties and is given by

\[ d_{x,y} = g_c \cdot \frac{r_y^2 r_x}{l_x(l_x r_x^2 + l_y r_y^2)} \cdot (V_y - V_x) \] (2.18)

where \( g_c \) (in mS/cm\(^2\)) is the strength of the coupling, \( l_d \) and \( l_s \) are the dendritic and somatic lengths (always 1 \( \mu m \)), and \( r_d \) and \( r_s \) are the dendritic and somatic radii.

In the model, the properties of the sodium conductance, \( g_{Na} \), and the delayed-rectifier potassium conductance, \( g_{K_r} \), are based on known features of the overall spiking activity of DA neurons. The results shown in Figure 2.2 were obtained with gating kinetics for the activation/inactivation of the sodium and delayed rectifier potassium conductances

\[
\alpha_m(V) = -0.32 \cdot \frac{(V + 33)}{\exp\left(\frac{-(V + 33)}{4.5}\right) - 1}, \quad \beta_m(V) = \frac{0.28 \cdot (V + 4)}{\exp\left(\frac{V + 4}{10.4}\right) - 1}, \quad (2.19)
\]

\[
\alpha_h(V) = 0.0196 \cdot \exp\left(\frac{-(V + 47)}{18}\right), \quad \beta_h(V) = \frac{2.45}{1 + \exp\left(\frac{-(V + 24)}{4}\right)}, \quad (2.20)
\]

\[
\alpha_n(V) = -0.3584 \cdot \frac{(V + 2)}{\exp\left(\frac{-(V + 2)}{3}\right) - 1}, \quad \beta_n(V) = 0.56 \cdot \exp\left(\frac{-(V + 20)}{5.8}\right) \quad (2.21)
\]

in addition to adjusting the voltage half-activation, \( V_{HK} \), and sensitivity, \( V_{SK} \), for the slow potassium conductance as shown in the caption of Figure 2.2.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value and units</th>
<th>Biological description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$</td>
<td>$1 \mu F/cm^2$</td>
<td>Membrane capacitance</td>
</tr>
<tr>
<td>$\bar{g}_{Ca,L}$</td>
<td>$0.20 mS/cm^2$</td>
<td>Maximal conductance for L-type calcium current</td>
</tr>
<tr>
<td>$E_{Ca}$</td>
<td>$100 mV$</td>
<td>Reversal potential for calcium ions</td>
</tr>
<tr>
<td>$\bar{g}_K$</td>
<td>$0.4 mS/cm^2$</td>
<td>Maximal potassium conductance</td>
</tr>
<tr>
<td>$V_{HK}$</td>
<td>$20 mV$</td>
<td>Half-activation voltage for the potassium conductance</td>
</tr>
<tr>
<td>$V_{SK}$</td>
<td>$13.8 mV$</td>
<td>Activation sensitivity for the potassium conductance</td>
</tr>
<tr>
<td>$\bar{g}_{K(Ca)}$</td>
<td>$0.3 mS/cm^2$</td>
<td>Maximal calcium-dependent potassium conductance</td>
</tr>
<tr>
<td>$K_{Ca}$</td>
<td>$250 nM$</td>
<td>Half-activation calcium concentration</td>
</tr>
<tr>
<td>$E_K$</td>
<td>$-90 mV$</td>
<td>Reversal potential for potassium</td>
</tr>
<tr>
<td>$g_L$</td>
<td>$0.05 mS/cm^2$</td>
<td>Leak conductance</td>
</tr>
<tr>
<td>$E_L$</td>
<td>$-50 mV$</td>
<td>Leak reversal potential</td>
</tr>
<tr>
<td>$\beta$</td>
<td>$0.05$</td>
<td>Ratio of free to buffered calcium</td>
</tr>
<tr>
<td>$r_s$</td>
<td>$10 \mu m$</td>
<td>Soma radii</td>
</tr>
<tr>
<td>$r_d$</td>
<td>$0.5 \mu m$</td>
<td>Dendritic radii</td>
</tr>
<tr>
<td>$z_{Ca}$</td>
<td>$2$</td>
<td>Valance of calcium</td>
</tr>
<tr>
<td>$P_{Ca}$</td>
<td>$0.25 nm/s$</td>
<td>Maximum rate of $Ca^{2+}$ transport by the pump</td>
</tr>
<tr>
<td>$\bar{g}_{Na}$</td>
<td>$150 mS/cm^2$</td>
<td>Maximal conductance for sodium current</td>
</tr>
<tr>
<td>$E_{Na}$</td>
<td>$55 mV$</td>
<td>Reversal potential for sodium</td>
</tr>
<tr>
<td>$\bar{g}_{K_s}$</td>
<td>$4 mS/cm^2$</td>
<td>Maximal conductance delayed-rectifier potassium current</td>
</tr>
<tr>
<td>$n_d$</td>
<td>$10$</td>
<td>Number of small identical dendritic compartments attached to the soma</td>
</tr>
<tr>
<td>$g_c$</td>
<td>$2.5 mS/cm^2$</td>
<td>Strength of the coupling</td>
</tr>
<tr>
<td>$l_s$</td>
<td>$1 \mu m$</td>
<td>Somatic length</td>
</tr>
<tr>
<td>$l_d$</td>
<td>$1 \mu m$</td>
<td>Dendritic length</td>
</tr>
</tbody>
</table>

Table 2.1: Parameter values for the coupling and spike producing currents of the two-compartment DA model. Values are derived from the model of Kuznetsov et al. (2006).

Figure 2.2 shows simulation results for a dendritic compartment representing 10 dendritic segments of $1 \mu m$ diameter connected to a $20 \mu m$ diameter soma where the membrane potentials in the soma and the dendrites are different throughout most of the cycle. Experimental results suggest that the generation of burst firing at rates higher than 10 $Hz$ can be obtained via dendritic applications of NMDA. In order to
explore this hypothesis, the voltage dependent synaptic current from \(\text{NMDA}\) receptors, equation (2.1) with conductance defined by equation (2.17), is added to the balance of currents that make up the pacemaking firing observed in Figure 2.2. Kuznetsov et al. (2006) obtain bursting with gating kinetics for the activation/inactivation of the spike generating currents that are adjusted to

\[
\alpha_m(V) = \frac{-0.32 \cdot (V + 31)}{\exp\left(-\frac{(V + 31)}{4}\right) - 1}, \quad \beta_m(V) = \frac{0.28 \cdot (V + 4)}{\exp\left(\frac{V + 4}{5}\right) - 1}, \quad (2.22)
\]

\[
\alpha_h(V) = 0.01 \cdot \exp\left(-\frac{(V + 47)}{18}\right), \quad \beta_h(V) = \frac{1.25}{1 + \exp\left(-\frac{(V + 24)}{5}\right)}, \quad (2.23)
\]

\[
\alpha_n(V) = \frac{-0.0032 \cdot (V + 5)}{\exp\left(-\frac{(V + 5)}{10}\right) - 1}, \quad \beta_n(V) = 0.05 \cdot \exp\left(-\frac{(V + 10)}{16}\right). \quad (2.24)
\]

Figure 2.3 demonstrates simulation results for a 500 ms application of \(\text{NMDA}\) to the dendrites which produces a period of high-frequency oscillation superimposed on the slow spontaneous regular spiking. Parameters are given in Tables 2.1 and 2.2.
Figure 2.3 suggests that the fast calcium oscillation in the dendrite is increased by *NMMA* activation. In addition, Kuznetsov et al. (2006) conclude that during the fast oscillation, action potentials are initiated in the dendrite and propagate to the soma hinting that during the application of NMDA, the roles of the dendrite and the soma are reversed.

Figure 2.3: Replica of Kuznetsov et al. (2006) figures 8A and 8B. NMDA induced bursting in the 2 compartment spiking model used in Figure 2.2. NMDA (0.4 mS/cm$^2$) is applied for 500 ms starting at 600 ms. The left panel shows the slow background firing rate is dominated by the soma. During the application of NMDA, firing rapidly increases to approximately 16 Hz. The right panel shows that during the rapid firing, somatic calcium increases slowly with each action potential, contributing smaller calcium increments than those seen during spontaneous firing. Dendritic calcium transients remain large throughout high-frequency firing. Parameters as in Table 2.1 and 2.2, except $V_{HK} = -10$ mV, $V_{SK} = 7$ mV, $g_{Ca,L} = 0.15$ mS/cm$^2$ and $g_c = 0.3$ mS/cm$^2$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value and units</th>
<th>Biological description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$g_{NMDA}$</td>
<td>0.4 mS/cm$^2$</td>
<td>Maximal conductance for NMDA current</td>
</tr>
<tr>
<td>$[Mg^{2+}]_o$</td>
<td>1.4 mM</td>
<td>Extracellular $Mg^{2+}$ concentration</td>
</tr>
<tr>
<td>$E_{NMDA}$</td>
<td>0 mV</td>
<td>Reversal potential for the NMDA current</td>
</tr>
</tbody>
</table>

Table 2.2: Parameter values for the NMDA current for the two-compartment DA model. Values are derived from the model of Kuznetsov et al. (2006).
MATHEMATICAL BACKGROUND: MODELS OF NEUROTRANSMISSION

In this chapter we present a brief history of models of the biochemical cascade leading to DA exocytosis known as the turnover process. We discuss how these models explain and probe the nature of the dynamic process by answering specific physiological questions.

3.1 Functional organization models

Functional organization models describe processes that achieve their regulation through the interaction of chemical reactions, storage, and transport. They represent metabolic processes in which the flux of the material from one compartment to another can be assumed to depend, linearly or nonlinearly, on the mass or concentration of material in the source compartment only. The model consists of writing mass balance equations for each compartment in terms of material flowing into that compartment and the flux of material from that compartment to other sites (Carson et al., 1983). In general the dynamics of the $i^{th}$ compartment can be described by

$$\frac{dQ_i}{dt} = R_{i0} + \sum_{j=1,j \neq i}^{n} R_{ij}(Q_j) - \sum_{j=1,j \neq i}^{n} R_{ji}(Q_i) - R_{0i}(Q_i), \quad (3.1)$$

where $Q_i$ describes the quantity of material in compartment $i$ where $i \in \{1,2,...,n\}$. The flux of material into compartment $i$ from compartment $j$, depending on $Q_j$ only, is characterized by $R_{ij}$. Finally, the flux of material into/from compartment $i$ from/into the external environment are explained by $R_{i0}$ and $R_{0i}$, respectively. In some cases, fluxes may be modeled using a linear dependence, $R_{ij} = k_{ij}Q_j$, where $k_{ij}$ is a constant defining the fractional rate of transfer of the material into compartment $i$ from compartment $j$. In others, Michaelis-Menten dynamics are required so that $R_{ij} = \frac{\alpha_{ij}Q_j}{\beta_{ij} + Q_j}$, where $\alpha_{ij}$ is the saturation value of the flux $R_{ij}$, and $\beta_{ij}$ is the value of $Q_j$ at which $R_{ij}$ is equal to

21
half of its maximal value.

### 3.2 Models of dopamine functional organization

Björklund and Dunnett (2007b) present evidence that the discovery of DA as a neurotransmitter in its own right in 1958 by Carlsson (Carlsson, 2002) did not attract much interest in the research community until 1967 when Cotzias and colleagues made a breakthrough in L-DOPA therapy (Cotzias et al., 1967). This turning point in DA research was soon met by modeling approaches to characterize the functional properties of DA neurons. The pioneering work presented in Doteuchi et al. (1974) models DA synthesis through an ordinary differential equation that quantifies the changes in cytoplasmic DA concentration. They model the change in the cytosolic DA concentration, $[DA]_c$, as the difference between the rate of synthesizing DA, $k_{dopa} \cdot [dopa]$, and the rate that cytoplasmic DA is packed into vesicles, $k_{DA} \cdot [DA]_c$ so that

$$d[DA]_c = k_{dopa}[dopa] - k_{DA}[DA]_c.$$ (3.2)

Costa et al. (1975) report that “if maintenance of DA neuronal function depends on the constancy of the DA concentrations, and if the latter is the reflection of a dynamic steady state, then the quantitative study of this dynamic equilibrium indeed probes a fundamental aspect of neuronal function.” Thus, understanding the transient properties exerted by the changes in the functional organization of DA neurons is crucial to understanding the possible effects involved in the change in cytoplasmic concentration of DA after neuronal damage.

Mathematical models of the functional organization of DA neurons usually involve some aspects of the specific steps in the turnover processes. The models can be classified as DA synthesis models, DA vesicular-release models, DA overflow models and its derivations, DA feedback control models and DA synapse models. Details for
these different model categories are presented below.

**Dopamine synthesis models**

The first step in the turnover process is the synthesis of DA. The pathway for the synthesis of DA from tyrosine is divided into two steps. The first, a rate limiting step, is the catalyzation of the hydroxylation of tyrosine by the enzyme tyrosine hydroxylase (TH) to L-DOPA involving biopterin as its cofactor. The second is the catalyzation of L-DOPA by L-amino decarboxylase (AADC; DOPA decarboxylase) to DA (Sabban, 1996). For a more complete description of the synthesis process, please refer to Cooper et al. (2003) or Figure 1.1.

Available models of DA synthesis focus on (i) the core reaction that converts DOPA to DA with modifications due to competitive inhibitors (Best et al., 2009; Porenta and Riederer, 1982), cofactors (Best et al., 2009; Justice et al., 1988; Porenta and Riederer, 1982) and various transport processes (Best et al., 2009; Justice et al., 1988; Nicolaysen et al., 1988; Nicolaysen and Justice, 1988; Porenta and Riederer, 1982) using a Michaelis-Menten kinetic approach, (ii) the conversion of DOPA to DA (Doteuchi et al., 1974; King et al., 1984) to calculate the DA turnover rate (equation 3.2) (Doteuchi et al., 1974) or (iii) the complete synthesis process (Best et al., 2009; Tuckwell, 1993, 1994, 2007). These models all assume the existence of TH and AADC in midbrain neurons. Ikemoto et al. (1998) report that the existence of TH and AADC are not homogeneous among midbrain neurons, having found evidence for the existence of \( TH^+/AADC^- \) and \( AADC^+/TH^- \) neurons in the midbrain area. This raises the possibility of the existence of DA neurons without TH or with so little TH that it is virtually undetectable in the cell (Björklund and Dunnett, 2007a; Ikemoto et al., 1998).

**Dopamine vesicular-release models**

DA is synthesized at release terminals (Harsing Jr, 2008), and newly synthesized DA resides in the release terminal’s cytoplasmic pool where DA is stored in vesicles by
the vesicular monoamine transporter (VMAT). Upon arrival of action potentials, the re-
lease terminal membrane is depolarized, evoking ion dependent mechanisms that trig-
ger vesicles containing DA to dock with the presynaptic membrane causing DA release
(Binder et al., 2001; Harsing Jr, 2008). See Figures 1.1 and 4.1. Mathematical mod-
els for vesicular release mechanisms usually are frequency-dependent synaptic models
that study the dynamics of the vesicular cycle. A general assumption used in these
types of models is that vesicles are in one of the following three states: available (the
reserve pool), active (readily releasable pool), or recovering (pool of empty vesicles)
(Axmacher et al., 2004; Catllá et al., 2008). These models usually focus on release
(Axmacher et al., 2004; Bertram, 1997) that is evoked by a short depolarization such as
that caused by an action potential (Bertram, 1997). They have been used to infer how
terminative changes in extracellular DA cause alterations in vesicular DA and vesicle dynamics
(Axmacher et al., 2004).

_Dopamine overflow models_

Experimental studies of DA release are usually done through electrical stimulation of
the medial forebrain bundle, which causes DA overflow in the ECS. DA overflow
models originated with the modeling work of Wightman et al. (1988). Their single
compartment model examines extracellular DA concentration gradients after electrical
stimulation of the medial forebrain bundle. The neurochemical model is represented
schematically in Figure 2 of Wightman and Zimmerman (1990) and by the equation

\[
d\left[DA\right]_e dt = \frac{1}{\tau} \cdot \left[DA\right]_p - \frac{V_{max} \cdot \left[DA\right]_e}{K_m + \left[DA\right]_e}
\] (3.3)

where \([DA]_e\) is the instantaneous concentration in the ECS, \([DA]_p\) is the concentration
of DA release per stimulus, \(\tau\) is the time between pulses (reciprocal of the stimulation
frequency), \(K_m\) is the Michaelis constant for DA uptake, and \(V_{max}\) is the maximal rate
of uptake of DA. Originally, this model was used to explain results concerning factors
that regulate DA extracellular concentrations in the rat neostriatum over a broad range of conditions (May et al., 1988; Wightman et al., 1988). However, it has been used to understand (Garris et al., 1994, 1997; Greco et al., 2006; Jones et al., 1995; Kennedy et al., 1992; Wightman and Zimmerman, 1990; Wu et al., 2001, 2002) and to perform statistical analysis (Bergstrom and Garris, 2003) of several other experiments, as well as, to study neurological disorders such as attention deficit hyperactive disorder (Viggiano et al., 2004). In particular, equation (3.3) appears in a series of articles studying DA overflow in ECS induced by electrical stimulation (Garris et al., 1994, 1997; Greco et al., 2006; Jones et al., 1995; Kennedy et al., 1992; Wightman and Zimmerman, 1990; Wu et al., 2001, 2002). Moreover, the model has been used to study changes in extracellular DA concentration due to the administration of pharmacological agents (Jones et al., 1995; Kennedy et al., 1992; Wightman and Zimmerman, 1990; Wu et al., 2001), the control of presynaptic autoreceptors of DA neurotransmission (Wu et al., 2002), and the efflux of DA from the axonal terminal to distal receptors (Garris et al., 1994).

From a more statistical point of view, using equation (3.3), Bergstrom and Garris (2003) study the correlation between firing rate, DA release, and DA uptake in control rats and following 6-OHDA lesions. Viggiano et al. (2004) used equation (3.3) to study the effects of methylphenidate, a stimulant used to treat patients with attention deficit hyperactive disorder.

Although, equation (3.3) agrees with experimental data, this model has been heavily criticized (Chen, 2005a) and does not display changes in extracellular DA uptake kinetics seen in in vivo experimental data obtained by other intracerebral measurement methods (Schönfuss et al., 2001).
Equation (3.3) has served as the stepping stone for a series of new models focusing on analyzing the electrically evoked DA kinetics in the rat neostriatum (Chen and Budygin, 2007), the dynamical changes in spike production and DA release (Montague et al., 2004), the basal extracellular DA concentration in the striatum (Michael et al., 2005), and the microdialysis zero-net-flux method used to estimate extracellular DA concentration (Chen, 2005a,b). However, all of these models have a shortcoming. As described in Chapter 1, DA neurons communicate mainly via volume transmission since most DA released is in a nonsynaptic form. Thus, modeling DA concentration may require including diffusion as well as the reuptake process exhibited in the ECS (Nicholson, 2001).

The first model of this type, Nicholson’s diffusion-uptake model, appeared in 1995. This new model was an attempt to quantify the dynamics exerted by DA neurons at the ECS and is represented by

\[
\frac{\partial [DA]_e(x,t)}{\partial t} = D \cdot \frac{\partial^2 [DA]_e(x,t)}{\partial x^2} - \frac{V_{max} \cdot [DA]_e(x,t)}{K_M + [DA]_e(x,t)},
\]

(3.4)

where \([DA]_e(x,t)\) is the concentration of diffusing DA as a function of distance, \(x\), from the ECS. \(D\) is the diffusion coefficient and \(K_M\) and \(V_{max}\) are the Michaelis constant and the maximal velocity constant for reuptake, respectively.

Nicholson’s diffusion-uptake model (Nicholson, 1995) has been the foundation of many models that examine extracellular DA concentration through analytical and numerical solutions which are used to evaluate the efficacy of the different methods used to measure extracellular DA kinetics in the neostriatum (Cragg et al., 2001; Lu et al., 1998; Nicholson, 1995; Peters and Michael, 1998, 2000; Schönfuss et al., 2001).
In addition, recent research efforts have shown the model to be useful for predicting volume transmission (Venton et al., 2003) and its link to neural activity (Dreyer et al., 2010; Thivierge et al., 2007). Based on equation (3.3), Schmitz et al. (2001) present a one-dimensional random walk model of DA diffusion in the ECS where computational studies are used to investigate the hypothesis that amphetamine mediates redistribution of vesicular DA to the cytoplasmic pool.

Dopamine feedback control models

Changes in extracellular DA concentration are maintained, for the most part, through interactions among extracellular degradation, diffusion, receptor activation (negative feedback), release, and reuptake. Mathematical and computational models of DA feedback control focus on receptor activation, release, and reuptake (Dreyer et al., 2010; Koshkina, 2006; Porenta and Riederer, 1982; Tretter and Eberie, 2002). These models have been used to evaluate and test receptor contributions to DA extracellular concentration gradients under pharmacological agents (Koshkina, 2006), depict the time course of transmitter release, binding to receptors, and reuptake (Tretter and Eberie, 2002), or as part of a larger scale model which describes the presynaptic biochemical cascade leading to exocytosis (Porenta and Riederer, 1982). This will be described more completely in the next sections.

Dopaminergic synapse models

The models described in this section have been developed to describe the functional organization of the turnover process, but none actually includes all six steps. One of the first mathematical models of the complete DA synapse was reported in Porenta and Riederer (Porenta and Riederer, 1982) and was used in the analysis of human DA dynamics in Parkinson’s disease and the aging process. The model was intended to characterize the dynamic equilibrium exerted during the turnover process as described in Figure 1.1 and represented schematically in Figures 1 and 2 of Porenta and Riederer (1982). The equations are
where \( x_i \) for \( i = 1, \ldots, 6 \) represent the instantaneous concentration of DOPA, cytosolic DA (free DA), bound DA (considered to be inside a vesicle and treated as a readily releasable pool), released DA, and competitive TH inhibitors I1 and I2, respectively. The constants \( k_{ij} \) are rate constants, \( c_{ij} \) are binding constants, \( v_i \) are the maximal values associated with \( c_{ij} \), and \( a_1 \) and \( a_2 \) are constant concentrations for tyrosine and its cofactor, respectively.

Although the model may be considered to describe a single nerve terminal, in reality, the model represents the average behavior of a population of DA nerve terminals. Equations (3.5) - (3.10) provide the foundation for a more comprehensive model of the DA presynaptic terminal, which was reported in Justice et al. (1988). This model includes DA synthesis, storage, release, uptake, and metabolism. The model is represented schematically by Figure 1 in Justice et al. (1988) and by
\[
\frac{d\text{DOPA}}{dt} = \frac{V_{mTH}}{1 + \left(\frac{K_{mTYR}}{TYR}\right)\left(1 + \frac{K_{mCOF}}{COF}\right)\left(1 + \frac{k_{fDA}}{fDA}\right)} - k_{DC\text{DOPA}} \tag{3.11}
\]
\[
\frac{d\text{bDA}}{dt} = k_{DC\text{DOPA}} + \frac{V_{mf}}{1 + \frac{K_{mfl}}{fDA}} - \frac{V_{mfb}}{1 + \frac{K_{mfb}}{fDA}} - k_{bDA} - k_{ibDA} + k_{fbDA} \tag{3.12}
\]
\[
\frac{d\text{fDA}}{dt} = \frac{V_{mfr}}{1 + \frac{k_{mftr}}{fDA}} - \frac{V_{mfr}}{1 + \frac{k_{mfbr}}{fDA}} - \frac{V_{mfr}}{1 + \frac{k_{mfbr}}{fDA}} - k_{maof\text{fDA}} \tag{3.13}
\]
\[
\frac{d\text{rDA}}{dt} = k_{ib\text{bDA}} + \frac{V_{mrf}}{1 + \frac{k_{mfr}}{rDA}} - \frac{V_{mrf}}{1 + \frac{k_{mfr}}{rDA}} - \frac{V_{mrf}}{1 + \frac{k_{mfr}}{rDA}} \tag{3.14}
\]
\[
\frac{d\text{gDA}}{dt} = \frac{V_{mrg}}{1 + \frac{k_{mrg}}{gDA}} - k_{maog\text{gDA}} \tag{3.15}
\]
\[
\frac{d\text{iDA}}{dt} = k_{ib\text{bDA}} - k_{ib\text{iDA}} \tag{3.16}
\]
\[
\frac{d\text{3-MT}}{dt} = \frac{V_{mrm}}{1 + \frac{k_{mrm}}{3-MT}} - k_{maom3-MT} \tag{3.17}
\]
\[
\frac{d\text{DOPAC}}{dt} = k_{maof\text{fDA}} + k_{maog\text{gDA}} - k_{contd\text{DOPAC}} - k_{clh\text{DOPAC}} \tag{3.18}
\]
\[
\frac{d\text{HVA}}{dt} = k_{contd\text{DOPAC}} + k_{maom3-MT} - k_{clh\text{HVA}} \tag{3.19}
\]

where the variables represent the instantaneous concentrations of DOPA, readily releasable vesicular DA (bDA), cytosolic DA (fDA), extracellular DA (rDA), glial DA (gDA), inactive vesicular DA (iDA), 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). The constants \(k_{ij}\) are rate constants, \(K_{mi j}\) are binding constants, \(V_{mi j}\) are the maximal rate constants associated with \(K_{mi j}\), and \(TYR\) and \(COF\) are constant for the tyrosine and the cofactor concentrations respectively.

Equations (3.11) - (3.19) have been used to study DA concentration in the ECS after electrical stimulation of the medial forebrain bundle in rat striatum (Justice et al., 1988; Nicolaysen et al., 1988; Nicolaysen and Justice, 1988). They were also used to study the effects of current and frequency on a population of MDNs (Nicolaysen et al., 1988) and the effects of cocaine on DA uptake and release (Nicolaysen and Justice, 1988). Although, equations (3.5) - (3.10) and (3.11) - (3.19) appear to be very similar,
there are several differences in their content and their interpretation. The three main differences between the systems are that equations (3.11) - (3.19) exclude the receptor mediated regulation of the extracellular DA concentration control process and include a reserve vesicular pool in addition to the readily releasable vesicular pool. The differences between the vesicular pools may be that readily releasable vesicular DA is simply closer to the neuronal membrane than the reserve vesicular pool, and thus has a greater access and consequently a greater probability of release. Finally, the vesicular storage process in equations (3.6) and (3.7) is described as a linear process while equations (3.12) and (3.13) describe it as a Michaelis-Menten catalyst-assisted reaction. Note that the processes by which DA molecules are transported are difficult to describe mathematically because of the complex geometry. Perhaps for this reason the phenomenological representation of the process of DA transporters in compartmental models are, for the most part, characterized by Michaelis-Menten dynamics. Quantifying the process of DA transport will be explored further in Chapter 4.

Using techniques from biochemical systems theory, Qi et al. (2008a,b, 2009, 2010) recently presented a mathematical model of DA homeostasis including the changes exerted by genetic, environmental and pharmacological factors. Their model integrates metabolites, enzymes, transports, and regulators involved in the turnover process as shown in Figure 2 of Qi et al. (2008b). The model includes greater detail than that in either Justice et al. (1988) or Porenta and Riederer (1982), and also assesses factors implicated in the pathogenesis and treatment of Parkinson’s disease (Qi et al., 2008a) as well as schizophrenia (Qi et al., 2008b).

3.3 Models of dopamine neurotransmission

The mechanisms involved in DA neurotransmission are greatly affected by homeostatic influences (Grace, 1991). A correspondence between DA cell firing activity and DA turnover is supported by experimental studies (Grace, 1991) and modeling approaches
The first mathematical model of nigrostriatal DA dynamics involved DA synthesis, storage, release, uptake, metabolism and firing activity (King et al., 1984). This model consists of equations

\[
\begin{align*}
\dot{x} &= \delta - x - \beta_1 y_1 - \beta_2 y_2 \quad (3.20) \\
\dot{y}_1 &= \alpha M x - \rho y_1 \quad (3.21) \\
\dot{y}_2 &= T x - \rho y_2 \quad (3.22) \\
\dot{M} &= \overline{\phi}(x) - dM \quad (3.23) \\
\overline{\phi}(x) &= \begin{cases} 
A + B(x - \overline{x})^2 & \text{if } x < 2\overline{x} \\
A + B \cdot \overline{x}^2 & \text{if } x > 2\overline{x}
\end{cases} \quad (3.24)
\end{align*}
\]

where the variables \(x, y_1, y_2,\) and \(M\) represent the firing rate of the DA neuron, the postsynaptic concentration of striatal released DA, the nigral concentration of released DA, and the concentration of functional synaptic stores, respectively. The constant \(\delta\) is the external depolarizing input to substantia nigra DA cells, \(\beta_1\) is the long-loop striatonigral feedback constant (proportional to the postsynaptic receptor number), \(\beta_2\) is the short-loop nigral dendrodendritic feedback constant (proportional to the presynaptic receptor number), \(\rho\) is the reuptake rate of DA, \(T\) is the nigral DA released per impulse, \(\alpha\) is the variable proportional to the release rate and the equilibrium constant for the synaptic stores of DA, and \(d\) is the degradative turnover rate of the functional DA in the synaptic stores. Finally, the model also contains a function \(\overline{\phi}(x)\) that describes the striatal synthesis of DA as a function of the firing rate.

Equations (3.20) - (3.24) help characterize the firing activity of DA neurons as exerting chaotic behavior using a difference logistic model. An analysis of the firing rates in the nigrostriatal (King et al., 1984) and mesolimbic DA systems (Shaner, 1999) based on the model’s response helped establish hypotheses concerning mechanisms by
which excessive DA transmission could produce psychotic symptoms in schizophrenics and the shift behavior known as “on-off phenomenon” in L-DOPA treated Parkinsonian patients.

3.4 Overview

The task of modeling the DA synapse requires some degree of biological detail, but as in any modeling approach, the question is: how much detail is necessary? Among existing models, some exhibit a great deal of biological detail and a considerable level of mathematical complexity as seen in Best et al. (2009) and Qi et al. (2008a,b, 2009, 2010). Others focus on some underline assumptions with a more feasible level of mathematical difficulty (King et al., 1984; Justice et al., 1988; Porenta and Riederer, 1982). Although, every modeling approach will always lack something or will be found not truly to predict the dynamics of the biological process of interest, a model is an efficient way to generate and explore hypotheses. In this work we focus on DA turnover homeostasis and the interactions between the DA neuron firing activity and DA homeostasis.
Chapter 4

MODELING THE TURNOVER PROCESS

Recall that in general, dopamine neurotransmission depends on multiple mechanisms including chemical transmission (synaptic and non-synaptic) and volume transmission (Venton et al., 2003; Vizi and Lendvai, 2008). During DA synaptic transmission, presynaptic electrical signals result in incremental increases in extracellular DA concentration, and subsequent receptor occupation results in electrical activity in the postsynaptic neuron (Tretter and Scherer, 2006). Extracellular DA dynamics due to synaptic transmission critically depend on (1) the amount of released DA which in turn is controlled by the number of vesicles that fuse with the plasma membrane and the release probability, (2) the extracellular DA clearance through reuptake, and (3) the extracellular DA clearance through degradation. Further, the neuronal firing which determines release can be affected by feedback signals emitted by autoreceptors (Sulzer, 2011; Tretter and Scherer, 2006; Weiner and Joel, 2002). See a schematic of these processes in Figure 4.1.

The pharmacological application of addictive drugs induces changes in DA neuron firing and turnover dynamics (Figure 1.1) (Sulzer, 2011). The spatial and temporal changes at release sites, the receptor dynamics, and the interaction among synthesis, storage, release, reuptake and receptor dynamics are believed to be key mechanisms for determining extracellular DA concentration levels that can be influenced by the application of addictive drugs such as amphetamine and cocaine (Garris et al., 1994; Wu et al., 2002). The goal of this chapter is to explore the dynamics of the release process through mathematical modeling. In chapter 5 we use the model to consider the implications of the application of cocaine.

The studies carried out in this chapter will be done at a representative single
cell level by developing and analyzing a mathematical model of the turnover process in DA neurons and interactions with DA neuron firing. The equations that describe the turnover process will target the six basic steps outlined in Figure 1.1 while the DA neuron firing that drives release will be modeled using the Kuznetsov et al. (2006) DA neuron model presented in section 2.3. In the simulations presented here, $I_{app}$ is varied in this model in order to obtain DA neuron firing of different frequencies as shown in Figure 4.2.

4.1 Modeling extracellular DA concentration in the striatum

The three major mechanisms mentioned above lead to a characterization of the dynamics of extracellular DA in the striatum given by the equation

$$\frac{d[DA]_e}{dt} = J_{rel} - J_{DAT} - J^o_{eda}$$  \hspace{1cm} (4.1)

where $J_{rel}$ represents the flux of calcium-dependent DA release from the cytosol, $J_{DAT}$ represents the unidirectional flux of DA from the extracellular to the intracellular compartment via the DA plasma transporter (DAT), and $J^o_{eda}$ is the outwards extracellular flux representing the combined degradation and diffusion rate by which DA clears from the ECS. See Figure 4.1 for a graphical description of DA dynamics at the ECS.

$J_{rel}$: Calcium-dependent DA release flux

When an action potential arrives at the nerve terminal, it induces membrane depolarization, causing the opening of voltage-gated ion channels. The probability of release of a DA storage vesicle in response to the nerve impulse depends on the conductance of calcium through N-type channels into the active zone (Rocchitta et al., 2005). Assuming that intracellular calcium concentration transients are identical at all DA release sites, we model intracellular calcium at the synapse as described in section 2.1 (Wang et al., 2011)
Figure 4.1: Diagram of extracellular DA dynamics. Upon arrival into release sites, action potentials lead to calcium influx. The increase in calcium concentration drives vesicles to fuse with the plasma membrane, causing the release of DA molecules into the extracellular space (ECS). Once in the ECS, the plasma membrane DA transporter (DAT) uses the movement of $Na^+$ and $Cl^-$ down their electrochemical gradients to drive the reuptake of DA molecules against the concentration gradient. Extracellular DA molecules that escape reuptake either bind to receptors or are metabolized.

$$\frac{d}{dt} [Ca^{2+}]_{i_{syn}} = \frac{2\beta}{r_{syn}} (\alpha I_N - P_{Ca_{syn}} \cdot ([Ca^{2+}]_{i_{syn}} - [Ca^{2+}]_{i_o}))$$  (4.2)

where $[Ca^{2+}]_{i_o}$ represents the average basal intracellular calcium concentration. The N-type (high-threshold, rapid inactivation) calcium current is modeled using a Hodgkin-Huxley-like formalism given by

$$I_N(V) = g_N \cdot d_N \cdot f_N \cdot (E_{Ca} - V_s).$$  (4.3)

The activation, $d_N$, and inactivation, $f_N$, gating variables in equation (4.3) are given in steady-state form with corresponding steady-state activation/inactivation functions.
\[ d_\infty(V_s) = \frac{1}{1 + \exp\left[ \frac{-(V_s - V_{HN_d})}{V_{SN_d}} \right]} \]  
(4.4)

\[ f_\infty(V_s) = \frac{1}{1 + \exp\left[ \frac{-(V_s - V_{HN_f})}{V_{SN_f}} \right]} \]  
(4.5)

The time scales for the activation, \( \tau_{dN} \), and inactivation, \( \tau_{fN} \), are treated as constants to reflect experimental data (McNaughton and Randall, 1997; Randall and Tsien, 1995).

Following the ideas in Lee et al. (2009) and assuming that calcium dependent DA release occurs within less than a millisecond after the \( Ca^{2+} \) channels open (Oheim et al., 2006), the flux of DA release (\( J_{rel} \)) from the cytosol is equal to the average release flux per vesicle (\( \psi \)) times the average number of vesicles in the readily releasable vesicle pool (\( n_{RRP} \)) multiplied by the release probability function, \( P_{rel} \left([Ca^{2+}]_{i_{syn}}\right) \). Hence, the flux of calcium-dependent DA release is given by

\[ J_{rel} = \psi \cdot n_{RRP} \cdot P_{rel} \left([Ca^{2+}]_{i_{syn}}\right). \]  
(4.6)

The average release flux per vesicle, \( \psi \) (in \( nM/ms \)), within a single synapse is

\[ \psi = D \cdot \frac{C_v}{\pi \cdot r_{syn}^2} \]  
(4.7)

where \( D \) represents the diffusion coefficient of DA in the striatum, \( C_v \) represents the average concentration of DA when occupying the volume of a single DA vesicle, and \( r_{syn} \) defines the radius of the synaptic bouton (Garris et al., 1994; Staal et al., 2004). The average concentration of DA, \( C_v \), is given by the amount of DA in a vesicle divided by the volume of the vesicle (Fall et al., 2002). In turn, the amount of DA in a vesicle is given by the number of DA molecules in a vesicle divided by Avogadro’s number, \( 6.022145 \times 10^{23} \) molecules/1 mol (Garris et al., 1994).
We assume that each vesicle contains 5000 molecules of DA (De la Fuente-Fernández et al., 2004; Bruns and Jahn, 1995). Thus, the average DA concentration, $C_v$, for the volume of a single DA vesicle with a 25 nm radius (Pickel et al., 1981) is given by

\[
C_v = \frac{5000 \, \text{DA molecules}}{\frac{4}{3} \pi (25 \, \text{nm})^3} \cdot \frac{1 \, \text{mol}}{6.022145 \times 10^{23} \, \text{molecules}} = 126.855751 \, \text{mM}. \tag{4.8}
\]

Furthermore, Rice et al. (1994) estimate that the diffusion coefficient of DA in the striatum, $D$, is around $2.7 \times 10^{-6} \, \text{cm}^2/\text{s}$. Hence, we calculate the average release flux per vesicle, $\psi$, to be

\[
\psi = \frac{(2.7 \times 10^{-6} \, \text{cm}^2/\text{s}) \times (126.855751 \, \text{mM})}{\pi (0.25 \, \mu m)^2} = 174,439,179 \, \text{nM/ms}. \tag{4.9}
\]

The release probability function, $P_{rel}(\lbrack \text{Ca}^{2+}\rbrack_{syn})$ is given as a fourth power Hill equation (Keener and Sneyd, 2009; Lee et al., 2009) of the calcium concentration

\[
P_{rel}(\lbrack \text{Ca}^{2+}\rbrack_{syn}) = P_{rel,max} \frac{\lbrack \text{Ca}^{2+}\rbrack_{syn}^4}{\lbrack \text{Ca}^{2+}\rbrack_{syn}^4 + K_{rel}^4} \tag{4.10}
\]

where $P_{rel,max}$ denotes the maximum release probability and $K_{rel}$ denotes the calcium sensitivity (Lee et al., 2009).

$J_{\text{DAT}}$: Unidirectional flux of DA

The DA transporter (DAT) located on the plasma membrane of dopaminergic neurons belongs to the family of $\text{Na}^+/\text{Cl}^-$ dependent neurotransmitter transporters that couples substrate transport to co-transport of $\text{Na}^+$ and $\text{Cl}^-$ and is indirectly driven by $\text{Na}^+/K^+$-ATP (Chen and Reith, 2004; Rudnick, 1998; Zhen et al., 2005). It is believed that DAT
co-transport two Na\(^+\) ions and one Cl\(^-\) ion per each DA molecule transported across the membrane. Hence, the stoichiometry of the DA uptake process, \(DA^+/Na^+/Cl^- = 1:2:1\), results in the net movement of two positive charges per DA molecule (Chen and Reith, 2003; Ingram et al., 2002). See Figure 4.1.

Mathematical models of the co-transport process, for the most part, use a carrier-mediated mechanism involving a carrier \(X\) that can form a binary complex with the solute \(i\). Assuming that the carrier \(X\) (a) is confined to the membrane; (b) can exist in the free or bound (\(iX\)) forms; (c) that the amount of the carrier per unit area is constant and (d) the association-dissociation reactions occur only at the membrane interface, the carrier mediated process exhibits saturation kinetics; i.e., the rate of the transport gradually approaches a maximum as the concentration of the solute transported by the carrier increases (Byrne et al., 1988; Schultz, 1980; Stein, 1989).

For the last three decades, modeling studies such as Porenta and Riederer (1982), Justice et al. (1988), Wightman and Zimmerman (1990), Best et al. (2009) and most recently Wang et al. (2011) have used a phenomenological model for the DA transporter based on the notion of carrier-mediated transport, using the unidirectional flux of DA from the extracellular to the intracellular compartment. Quantitatively, the model omits the co-substrates Na\(^{2+}\) and Cl\(^-\) because of the assumption that the concentration of these ions in the extracellular compartment are relatively constant (Schenk, 2002).

Hence, \(J_{DAT}\) is described by

\[
J_{DAT} = \frac{V_{eda, max} \cdot [DA]_e}{K_{EDA} + [DA]_e}
\]  

where \(V_{eda, max}\) (in nM/ms) denotes the maximal velocity obtained as the product of the kinetic constant for the movement of DA across the cell membrane, \(k_{cat}\), and the concentration of DAT. The experimentally determined parameter, \(K_{EDA}\) (in nM), rep-
represents the concentration of DA at half-maximal velocity (Byrne et al., 1988; Earles and Schenk, 1999; Schenk et al., 2005; Schultz, 1980). The strong similarity between equation (4.11) and the familiar Michaelis-Menten formulation of enzyme kinetics is due to the belief that carriers are enzyme like molecules that comprise part of the protein portion of the lipoprotein membrane adapted after the introduction of the carrier hypothesis (Byrne et al., 1988; Schenk et al., 2005; Stein, 1989). A derivation for equation (4.11) is given in Keener and Sneyd (2009).

\[
J_{eda}^o: \textit{Outward extracellular flux rate}
\]

Once in the ECS, DA molecules are transported back into the nerve by DAT, degraded by enzymes such as catechol-O-methyltransferase (COMT) or diffuse out from the synaptic cleft. In order to maintain a level of simplicity, we regard enzyme degradation and diffusion as a single act modeled by the outward flux rate

\[
J_{eda}^o = k_{comt} \cdot [DA]_e
\]  

(4.12)

where the term \( k_{comt} \) (in \( ms^{-1} \)) represents the rate at which extracellular DA is removed from the ECS.

\textit{Establishing parameters}

According to Ross (1991), most (>95%) of the DA released into the extracellular area is recycled back into the terminal bouton by DAT. Therefore, the parameter values for our simple model of extracellular DA given by equation (4.1) (listed in Table 4.1) were chosen so that during tonic firing (5.1 Hz, Figure 4.2) more than 95% of the released DA is recycled by DAT. See Figure 4.3. Available parameter values are either taken or derived from the literature. The parameter values for \( P_{Ca_{syn}}, g_N, \) and \( \tau_{dN} \) were loosely set to simultaneously fit many different data sets concerning extracellular DA (Ben-Jonathan and Hnasko, 2001; Gonon, 1997; Hyland et al., 2002; Schmitz et al., 2003; Schultz, 1998, 2007).
<table>
<thead>
<tr>
<th>Model</th>
<th>Value (units)</th>
<th>Biological description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_{syn} )</td>
<td>0.25 ( \mu m )</td>
<td>Synapse radii</td>
</tr>
<tr>
<td>( P_{Ca_{syn}} )</td>
<td>0.025 ( \mu m/ms )</td>
<td>Maximum rate of ( Ca^{2+} ) transport by the pump in the bouton</td>
</tr>
<tr>
<td>([Ca^{2+}]_{i_o})</td>
<td>100 nM</td>
<td>Baseline intracellular calcium concentration</td>
</tr>
<tr>
<td>( n_{RRP} )</td>
<td>10</td>
<td>Average number of ready releasable vesicles</td>
</tr>
<tr>
<td>( P_{rel,max} )</td>
<td>0.14</td>
<td>Maximum release probability</td>
</tr>
<tr>
<td>( K_{rel} )</td>
<td>700 000 nM</td>
<td>Calcium sensitivity of transmitter release</td>
</tr>
<tr>
<td>( g_N )</td>
<td>3.275 mS/cm²</td>
<td>Maximal N-type calcium conductance</td>
</tr>
<tr>
<td>( V_{HN_d} )</td>
<td>−4.5 mV</td>
<td>Half voltage activation for the calcium conductance</td>
</tr>
<tr>
<td>( V_{SN_d} )</td>
<td>5.2 mV</td>
<td>Activation sensitivity for the calcium conductance</td>
</tr>
<tr>
<td>( \tau_{d_N} )</td>
<td>0.001 ms</td>
<td>Time scale for the calcium activation</td>
</tr>
<tr>
<td>( V_{HN_f} )</td>
<td>−74.8 mV</td>
<td>Half voltage inactivation for the calcium conductance</td>
</tr>
<tr>
<td>( V_{SN_f} )</td>
<td>6.5 mV</td>
<td>Inactivation sensitivity for the calcium conductance</td>
</tr>
<tr>
<td>( \tau_{f_N} )</td>
<td>183 ms</td>
<td>Time scale for the calcium inactivation</td>
</tr>
<tr>
<td>( E_{Ca} )</td>
<td>100 mV</td>
<td>Reversal potential for calcium ions</td>
</tr>
<tr>
<td>( V_{eda,max} )</td>
<td>6 nM/ms</td>
<td>Maximal velocity for DAT</td>
</tr>
<tr>
<td>( K_{EDA} )</td>
<td>30 nM</td>
<td>Dopamine concentration at half maximal velocity</td>
</tr>
<tr>
<td>( k_{comt} )</td>
<td>0.0083511 ms⁻¹</td>
<td>Combined degradation and diffusion rate</td>
</tr>
</tbody>
</table>

Table 4.1: Parameter values for the extracellular DA compartment described in Section 4.1. Values are derived from the literature (Ben-Jonathan and Hnasko, 2001; Canavier and Landry, 2006; Drapeau and Blaustein, 1983; De la Fuente-Fernández et al., 2004; Gonon, 1997; Grace, 1991; Huff and Davies, 2002; Kuznetsov et al., 2006; McNaughton and Randall, 1997; Pickel et al., 1996; Randall and Tsien, 1995; Schmitz et al., 2003; Simon and Llinás, 1985; Staal et al., 2004; Südhof, 1995; Tsien, 2009; Venton and Wightman, 2003; Viggiano et al., 2004). The parameter values for \( P_{Ca_{syn}}, g_N, \) and \( \tau_{d_N} \) were chosen to reflect experimental data.
As shown in Figure 1 in Grace (1991), transient DA release depends on the firing of DA neurons. Most experimental studies of DA release utilize electrical and pharmacological stimulation of the medial forebrain bundle, which causes DA overflow in the ECS. The term overflow is used to describe the increments in extracellular DA concentration over time observed during stimulation (Garris and Wightman, 1995). According to some experimental and modeling studies, extracellular DA concentration is very sensitive to spike rate frequency and firing pattern (Best et al., 2009; Wightman et al., 1988). In addition, similar studies suggest that extracellular DA concentration grows exponentially as a function of spike frequency and saturates as a function of stimulus duration (Gonon, 1988; Wightman et al., 1988; Wightman and Zimmerman, 1990).

We explore these ideas by letting the action potentials that originate in the soma (equation 2.10) drive the extracellular DA dynamics (equation 4.1) at the nerve terminal, where the process is mediated by the dynamics of calcium in the buton. Numerical solutions for the extracellular DA compartment are computed by implementing the Gear’s algorithm (ode15s solver) found in Matlab (Mathworks).

Recall that in freely moving rats, MDNs spontaneously discharge action potentials that last on average 1.5 ms and have a spontaneous mean firing rate of 3.7 ± 1.5 Hz, with a range anywhere from 0.8 to 8 Hz (Gonon, 1988; Hyland et al., 2002). Simulation results show that under normal conditions, the Kuznetsov et al. (2006) DA neuron model is capable of reproducing spikes like those found in regular DA cells that fire spontaneously; see Grace and Bunney (1984b) for comparison. In addition, after the introduction of an applied current ($I_{app} = 1.4$), simulations show a natural burst-like frequency of 15.9 Hz with equally spaced action potentials. See Figure 4.2. The impulse-dependent results seen with the coupling of our extracellular DA model (equa-
tion 4.1) with the Kuznetsov et al. (2006) DA neuron model agree with five essential results found in the neuroscience literature.

1. Resting levels of intracellular calcium at release sites are approximately 0.1 µM and can rise to 5 – 10 µM upon arrival of an action potential (Ben-Jonathan and Hnasko, 2001).

2. The amount of DA release per pulse is approximately constant (Gonon, 1997; Schultz, 2007).

3. Total increases of extracellular DA last for about 200 ms after a single pulse (Schultz, 1998).

4. 95.5% of released DA is recycled by DAT (Ross, 1991).

5. Any change in extracellular DA concentration is cleared from the ECS before the arrival of the next action potential (Schmitz et al., 2003).

All of these features are replicated in Figure 4.3.

We also consider results for a 20 second simulation of regularly spaced action potentials at 14 Hz (shown in Figure 4.4) under the assumption that there are 10 vesicles in the readily releasable pool. The model suggests that the extracellular DA concentration increases to 52.2 nM; however, this result does not agree with the experimental results of Gonon (1988). Likewise, Figure 4.5 shows that, according to the model, if on average there are only 10 vesicles in the readily releasable pool during the higher frequency firing of a burst then the estimated extracellular DA concentration would be 85.09 nM, which is significantly below the 150 – 400 nM range proposed by Schultz (1998).

These results lead to an exploration of the numerical solutions for the extracellular DA compartment using different values for the average number of readily releasable
Figure 4.2: Simulation results for the Kuznetsov et al. (2006) model defined in Chapter 2. Left panel shows voltage oscillations in the soma at a 5.1 Hz frequency while the right panel shows oscillations at a 15.9 Hz frequency. The oscillation frequencies are determined by the applied current parameter, $I_{\text{app}}$. Parameters as in Table 2.1 except for $g_c = 5 \text{ mS/cm}^2$.

Figure 4.3: Simulation results showing oscillations in the dopaminergic terminal upon arrival of the action potentials shown in the left panel of Figure 4.2 (5.1 Hz frequency). The left panel shows that as intracellular calcium approaches a limit cycle, intracellular calcium concentration in the terminal bouton is near 6 nM after the arrival of an action potential. The right panel estimates that as extracellular DA approaches its limit cycle, the amount of DA release per pulse is around 33 nM of which 95.5% is recycled back into the cell by DAT. Parameters as in Table 4.1.
Figure 4.4: A computational realization of the experiment proposed by Gonon (1988). The experiment consists of stimulating a DA neuron for twenty seconds where the cell fires at a rate of $14 \, Hz$ ($I_{app} = 1.01$). Simulation results show that an increase in the duration of the stimulation leads the maximum amount of extracellular DA to stabilize at about $52.24 \, nM$. Parameters as in Table 4.1.

Figure 4.5: Simulation results showing oscillations in the dopaminergic terminal upon arrival of the membrane potential described in the right panel of Figure 4.2 (15.9 $Hz$). The left panel shows the calcium concentration in the terminal bouton in the $5 - 10 \, \mu M$ range as suggested by Ben-Jonathan and Hnasko (2001). The right panel shows that higher frequency firing during bursting behavior increases extracellular DA concentration into the range of $85.09 \, nM$ for the model which is significantly below the $150 - 400 \, nM$ range suggested by Schultz (1998). Parameters as in Table 4.1.
vesicles, $n_{RRP}$, since De la Fuente-Fernández et al. (2004) and Südhof (1995) suggest that at any given point there are 10 – 30 readily releasable vesicles per terminal. The results shown in Figure 4.6, show that if $n_{RRP} = 20$ during a burst then the estimated maximum value of extracellular DA concentration would be approximately 413 nM which is relatively close to the 150 – 400 nM range suggested by Schultz (1998).

![Figure 4.6: Simulation results showing oscillations in the dopaminergic terminal upon arrival of the membrane potential described in Figure 4.2. The two upper panels show results for $n_{RRP} = 20$ while the two lower panels show results for $n_{RRP} = 30$. The upper right panel shows that, when $n_{RRP} = 20$, the higher frequency firing during bursting behavior increases extracellular DA concentration into the range of 413 nM which is relatively close to the 150 – 400 nM range suggested by Schultz (1998). Parameters as in Table 4.1.](image)

In addition, as seen in the left panel of Figure 4.7, our impulse-dependent model for extracellular DA (equation 4.1) with $n_{RRP} = 30$ predicts that after a 20 seconds simulation of regularly spaced stimulations at 14 Hz, extracellular DA concentration significantly increases to maximum values of approximately 453 nM, a result inside
the 300 – 840 nM range found by Gonon (1988). Moreover, the right panel of Figure 4.7 shows that the simulations generated by equation (4.1) agree with Gonon (1988) and Wightman and Zimmerman (1990), since simulations at various firing frequencies generate a DA peak that grows exponentially. Finally, the numerical results show that an increase in the duration of the simulation leads to saturation as shown in the left panel of Figure 4.7.

4.2 Modeling intracellular DA concentration in the striatum

As mentioned at the beginning of this chapter, a qualitative analysis of extracellular DA concentration relies heavily on the quantitative analysis of receptor-mediated feedback based on experimental concentration response curves in which the concentration of DA at the receptor site is linked with the response in firing rate, synthesis, release and reuptake. According to Grace (2001), terminal autoreceptors are categorized into two groups: synthesis modulating autoreceptors and release modulating autoreceptors. Synthesis modulating autoreceptors regulate DA synthesis via the modulation of the enzyme tyrosine hydroxylase (TH), the rate limiting enzyme in DA synthesis (Snow
and Calne, 1997); while the release modulating autoreceptors system exerts a rapid and powerful down regulation of spike dependent DA release (Grace, 2001).

How do autoreceptors modulate the firing rate, synthesis and release of DA neurons? Evidence suggests that the preservation of basal DA concentration levels following midbrain lesions occurs due to an increased firing activity of surviving striatal neurons (Elsworth and Roth, 2004; Snow and Calne, 1997) and an increase in the impulse induced synthesis and release of DA by surviving MDNs in the striatum, even though the firing rate activity in the midbrain does not increase (Groves et al., 1998). As reported in Grace (1991), the mechanisms involved in DA neurotransmission are greatly affected by homeostatic influences, thus in order to investigate the interactions among firing rate, synthesis and release, we need to incorporate additional intracellular DA compartments to describe the dynamics found within the terminal bouton.

As described in Figure 1.1, intracellular DA is compartmentalized into two distinct pools within the terminal bouton. These intracellular compartments are the cytosolic and vesicular DA. Therefore, intracellular DA dynamics can be characterized as the sum of the flux differences between the cytosolic and vesicular compartment; that is

\[
\frac{d[DA]_i}{dt} = \frac{d[DA]_c}{dt} + \frac{d[DA]_v}{dt} \quad (4.13)
\]

where

\[
\frac{d[DA]_c}{dt} = J_{synt} + J_{DAT} - J_{VMAT} - J_{ida}^0 \quad (4.14)
\]

\[
\frac{d[DA]_v}{dt} = J_{VMAT} - J_{rel}. \quad (4.15)
\]

As observed in equations 4.14 and 4.15, intracellular DA dynamics are controlled by
two sources and two loss processes. The sources are the flux of synthesized DA in
the nerve terminal cytoplasm, $J_{syn}$, and the DA uptake flux from the extracellular DA
compartment, $J_{DAT}$, while the loss processes are the flux of DA released into the ex-
tracellular DA compartment, $J_{rel}$, and the DA metabolized flux by monoamine oxidase
(MAO), $J_{ida}$. See Figure 4.8 for a graphical description of intracellular DA dynamics
at a terminal bouton.

![Diagram of intracellular DA dynamics](image)

Figure 4.8: Diagram of intracellular DA dynamics. Action potentials generated at the
axon hillock propagate down the axon to a terminal bouton and stimulate tyrosine hy-
droxylation (TH) which generates a process that culminates in newly synthesized DA
at the cytosolic area (Cooper et al., 2003). Newly synthesized DA mixes with inward
transported DA from the extracellular area, and together these are packed into vesicles
by the vesicular monoamine DA transporter (VMAT). See Figure 4.10. DA is then
released in a spike-dependent manner through the activation of voltage-sensitive cal-
cium channels (Grace, 2002). Once in the extracellular space (ECS), DA is recycled
back into its nerve terminal by the plasma DA membrane transporter (DAT) through a
coupling of the translocation of two $Na^+$ ions and a $Cl^-$ ion, which results in the move-
ment of two positive charges of DA (Ingram et al., 2002). Extracellular DA molecules
that escape reuptake either bind to receptors or are metabolized (Harsing Jr, 2008).
DA synthesis originates from the concentration of tyrosine (TYR) located in the terminal bouton and is divided into two steps. Each of the steps depends on a specific enzyme that acts as a catalyst (an agent that increases the rate of a chemical reaction) for that step. The first, a rate limiting step, is the catalyzation of the hydroxylation of tyrosine by the enzyme tyrosine hydroxylase (TH) to L-DOPA involving biopterin as its cofactor. The activity of TH is regulated by a balance among cytosolic DA that acts as an end product inhibitor by competing with its cofactor, by extracellular DA that acts as an inhibitor via the binding with synthesis modulating autoreceptors located on the nerve terminals, and by neuronal activity as a stimulator (Harsing Jr, 2008; Meyer and Quenzer, 2004; Paquette et al., 2009; Snow and Calne, 1997). The second step in the synthesis process is the catalyzation of L-DOPA by L-amino decarboxylase (AADC; DOPA decarboxylase) to DA. Inside the terminal bouton, the activity of dopa decarboxylase is extremely high. Hence L-DOPA is converted into DA almost instantaneously (Feldman et al., 1997; Harsing Jr, 2008; Meyer and Quenzer, 2004; Sabban, 1996).

If we assume that after L-DOPA is produced, it is instantaneously transformed into DA, the two step synthesis process can be simplified into a single step from TYR to DA with velocity, $V_{\text{syn}}$, and kinetics obeying the production rate for L-DOPA. See Figure 4.9 for a graphical description and Porenta and Riederer (1982), Justice et al. (1988), Tuckwell (2007), and Best et al. (2009) for equations describing the behavior. Unlike these publications, we choose not to model the intermediate steps involved in DA synthesis. Thus we model the flux of synthesized DA by
\[ J_{\text{syn}} = \frac{V_{\text{syn}}}{1 + \frac{K_{TYR}}{[TYR]} \left( 1 + \frac{[DA]_c}{K_{i(cda)}} + \frac{[DA]_e}{K_{i(eda)}} \right)} \]  

(4.16)

where the parameters \( K_{TYR} \) and \([TYR]\) (both in nM) represent the concentration of TYR at half maximal velocity and the concentration of TYR found in the terminal bouton respectively. The effects of the end product cytosolic DA and extracellular DA inhibitors are to increase \( K_{TYR} \) by a factor of \( \left( 1 + \frac{[DA]_c}{K_{i(cda)}} + \frac{[DA]_e}{K_{i(eda)}} \right) \), thus decreasing the velocity of \( V_{\text{syn}} \). The inhibition constants (in nM) are given by \( K_{i(cda)} \) and \( K_{i(eda)} \).

Derivation of equations of this form are given in Keener and Sneyd (2009).

Figure 4.9: Diagram of the synthesis pathway for DA. Our model assumes that the rate of DA production obeys the kinetics of L-DOPA production. DA production is stimulated by action potential arrival while simultaneously inhibited by the extracellular DA via the activation of synthesis modulating autoreceptors and intracellular DA via end-product inhibition.

As stated earlier, the activity of TH is regulated by neuronal activity (Snow and Calne, 1997). Chen et al. (2003) propose a general mechanism linking neuronal stimulation to the synthesis of DA through an indirect cascade of events which starts
with the influx of $Ca^{2+}$ into the terminal bouton. We were unable to find specific data regarding $Ca^{2+}$ mediated activation of synthesis in DA neurons and hence employed a simple fourth power Hill equation to modify the velocity of synthesis, $V_{syn}$. The specific equation for $V_{syn}$ is given by

$$V_{syn}(\{Ca^{2+}\syn\}) = \frac{V_{syn,max} \cdot [Ca^{2+}]_{syn}^4}{K_{syn}^4 + [Ca^{2+}]_{syn}^4}$$

(4.17)

where the parameters for the calcium sensitivity, $K_{syn}$ (in nM), and the maximal velocity for synthesis, $V_{syn,max}$ (in nM/ms), are set to fit experimental data concerning extracellular DA.

$J_{VMAT}$: Storage of DA into the vesicular pool

Synthesized DA resides in the nerve terminal cytosolic pool where DA is moved into vesicles by the vesicular monoamine transporter (VMAT) to await release to the extracellular DA compartment (Snow and Calne, 1997). In the cytosol, DA is present in low concentrations (100 – 1000 nM) and is subject to metabolism by MAO (Liu and Edwards, 1997). Unlike the $Na^+ / Cl^-$ dependent plasma DA transporter (DAT), the mechanism that transports DA into the vesicles couples uptake to a proton electrochemical gradient ($\Delta \mu_{H^+}$) generated by $H^+$-ATPase (Fei and Krantz, 2009; Harsing Jr, 2008). See Figure 4.10 for a graphical description of the dynamics of the vesicular monoamine transporter (VMAT). Vesicular DA is stored at extremely high concentrations (0.5 – 0.6 M), approximately $10^3 – 10^5$ greater than the concentration in the cytosol (Ben-Jonathan and Hnasko, 2001; De la Fuente-Fernández et al., 2004; Elsworth and Roth, 2004). Following the approach taken in previous models, we model the process of transporting DA molecules into vesicles as a bidirectional flux that depends on the two DA concentrations (Axmacher et al., 2004; Best et al., 2009). Assuming that the process of transporting DA molecules into vesicles follows Michaelis-Menten kinetics (Justice et al., 1988; Best et al., 2009), that is it saturates with increasing cytosolic
DA concentration and leakage is given as a linear process dependent on vesicular DA (Axmacher et al., 2004; Best et al., 2009; Porenta and Riederer, 1982), we obtain

\[ J_{VMAT} = V_{cda,\text{max}} \cdot \frac{[DA]_c}{K_{CDA} + [DA]_c} - k_{vda}[DA]_v \]  

(4.18)

where \( K_{CDA} \) (in \( nM \)) represents the concentration of DA at half maximum velocity and \( V_{cda,\text{max}} \) (in \( nM/\text{ms} \)) denotes the maximal velocity for the movement of DA into the vesicles. Simulations of the model using equation 4.18 indicate that under normal conditions, the rate at which vesicular DA leaks back into the cytosol, \( k_{vda} \) (in \( \text{ms}^{-1} \)), is negligible. Thus, we initially model the process of transporting DA molecules into vesicles as

\[ J_{VMAT} = \frac{V_{cda,\text{max}} \cdot [DA]_c}{K_{CDA} + [DA]_c} \]  

(4.19)

The physical justification for the use of Michaelis-Menten kinetics for the upload of molecules into vesicles is based on the fact that transport energy increases with an increasing concentration gradient between axonal cytoplasm and vesicle interior. Thus the accumulation of transmitter molecules into vesicles is a self limiting process (Axmacher et al., 2004).

\( n_{RRP} \): Average number of readily releasable vesicles

Extracellular DA concentration depends on, \( J_{rel} \), the flux of calcium dependent DA release (equation 4.1). In turn, the reaction flux depends on the number of vesicles in the ready releasable pool \( (n_{RRP}, \text{equation 4.6}) \). Grace (2001) suggests that release modulating autoreceptors, located in the neuron terminal, exert a rapid and powerful down regulation on the spike-dependent DA release. Assuming that this regulation exerts a control on the number of vesicles in the readily releasable vesicle pool, we propose that \( n_{RRP} \) can be quantified by
Figure 4.10: Diagram of the vesicular monoamine transporter (VMAT) dynamics. Newly synthesized DA mixes with inward transported DA from the extracellular area, and together these are packed into vesicles by the vesicular monoamine DA transporter (VMAT) using a proton concentration component, $\Delta pH$, and an electrical component, $\Delta \psi$ of the proton gradient $\Delta \mu_{H^+}$, or they are metabolized by intracellular enzymes (Fei and Krantz, 2009).

\[
R_{RRP} = \frac{40}{\left(1 + \exp\left[\frac{-([DA]_v - [DA]_{vo})}{[DA]_v s}\right]\right) \left(1 + \exp\left[\frac{[DA]_e - [DA]_{Ra}}{[DA]_s}\right]\right)}.
\]  

(4.20)

where $[DA]_v$, (in nM) denotes the initial vesicular DA concentration, $[DA]_v$, (in nM) denotes the sensitivity to vesicular concentration, $[DA]_Ra$, (in nM) denotes the high-affinity state for DA binding to receptors, and $[DA]_s$, (in nM) denotes the binding sensitivity.

$J^o_{ida}$: Outward intracellular flux

Dopamine molecules in the intracellular compartment can be metabolized by MAOs. For simplicity, we model the outward intracellular flux as
where the term \( k_{mao} \) (in \( ms^{-1} \)) represents the rate at which cytosolic DA is removed from the cytosol.

4.3 A model of DA neurotransmission

In sections 4.1 and 4.2, we develop models for extracellular and intracellular DA dynamics, respectively. The coupling of these models via equation (4.20) generates a complete model for DA dynamics in a representative single DA neuron bouton. Our compartmental model consists of three compartments: cytosolic, vesicular and extracellular DA that form the basis of a new mathematical model of DA synthesis, storage, release and reuptake. Our model consists of three differential equations (equations 4.1, 4.14 and 4.15) given below

\[
\begin{align*}
\frac{d[DA]_c}{dt} & = J_{synt} + J_{DAT} - J_{VMAT} - J_{ida}^o \\
\frac{d[DA]_v}{dt} & = J_{VMAT} - J_{rel} \\
\frac{d[DA]_e}{dt} & = J_{rel} - J_{DAT} - J_{eda}^o
\end{align*}
\]

where
This model can be driven by action potentials generated by any model of membrane potential or even by experimentally induced depolarization voltage recordings that are used to generate calcium concentrations used here according to equations 4.2-4.5. As an example we use it in conjunction with an excitable membrane model of a mesencephalic DA neuron (Kuznetsov et al., 2006). Our model has some similarity to the models found in Bertram (1997), Best et al. (2009), King et al. (1984), Lee et al. (2009), Porenta and Riederer (1982), Justice et al. (1988) and Tuckwell (2007), but most of these do not consider vesicle release to be calcium dependent, do not include the effects of release modulating autoreceptors on the number of vesicles in the readily releasable pool, and do not couple the model to a DA neuronal model simultaneously to study the qualitative changes in the dynamics of the entire system.

**Model assumptions**

In summary, based on the complexity of the DA system equilibrium and theoretical aspects of the modeling approach, our model has been developed under twelve major assumptions:

1. Membrane potentials that originate in the soma drive the intracellular calcium concentration at release sites via $I_N$, an N-type calcium current.
2. The model neglects the time delay due to action potentials propagate down the axon to the terminal at a rate of 0.5 m/s (Venton and Wightman, 2003).

3. Calcium dependent DA release occurs within less than a millisecond after the Ca^{2+} channels open and transient concentrations are identical in all DA release sites.

4. Dopamine is synthesized in the nerve terminal cytoplasm. If DA is synthesized in the cell body, then it undergoes axoplasmic transport to the terminal regions.

5. After L-DOPA is produced, it is almost instantaneously transformed into DA; hence, the two step synthesis process is simplified into a single step from TYR to DA with velocity, \( V_{syn} \).

6. The velocity at which TYR is converted into DA is dependent on intracellular calcium.

7. Cytosolic and vesicular DA are both treated as single compartments.

8. A vesicle contains 5000 DA molecules.

9. The rate at which vesicular DA leaks back into the cytosol, \( k_{vda} \), is neglegable.

10. Metabolization by MAO is regarded as linear.

11. Enzyme degradation and diffusion of extracellular DA are regarded as a single linear process.

12. Release modulating autoreceptors exert a control on the number of vesicles in the readily releasable pool.

**Establishing parameters**

The balanced regulation of cytosolic DA levels by synthesis, vesicular secretion, enzymatic breakdown, and extracellular reuptake is important since the availability of
cytosolic DA appears to be critical in a number of neurological disorders (Westerink, 2006). An in vivo study of unregulated cytosolic DA suggests that augmented levels of cytosolic DA lead to neurodegeneration (Chen et al., 2008). This study reinforces the idea that there is precise regulation among the processes that influence cytosolic DA concentration (Westerink, 2006).

As mentioned above, the concentration of cytosolic DA is in the range 100 – 1000 nM (Liu and Edwards, 1997). In addition, according to Eisenhofer et al. (2004) and Mosharov et al. (2003), VMAT sequesters between 90%-98% of cytosolic DA into storage vesicles. The parameter values for the intracellular DA compartment equations (4.14) and (4.15), listed in Table 4.2, were chosen so that during tonic firing (5.1 Hz, Figure 4.2) the average cytosolic DA concentration is around 1048.7 nM per cycle and 97% of the DA gained through synthesis and extracellular reuptake is sequestered into the vesicles per cycle. See Figure 4.11. Available parameter values are either taken or derived from the literature. The parameter values for $V_{\text{syn}, \text{max}}, K_{\text{syn}}, [DA]_v, [DA]_R$, and $k_{\text{mao}}$ were set to simultaneously fit many different data sets concerning intracellular and extracellular DA.

**Model validation**

Simulation studies were performed to verify that our model meets the experimental criteria previously mentioned in section 4.1. As in the earlier model, Figure 4.11 agrees with essential results found in the neuroscience literature. For example, experimental data suggest that resting levels of intracellular calcium at release sites are approximately 0.1 µM and can rise to 5 – 10 µM upon arrival of an action potential, the amount of DA release per pulse is approximately constant, increases of extracellular DA last for approximately 200 ms after a single pulse, 95.2% of released DA is recycled by DAT, and any change in extracellular DA concentration is cleared from the ECS before the arrival of the next action potential (Ben-Jonathan and Hnasko, 2001; Gonon, 1997;
Parameter | Literature values (units) | Biological description
--- | --- | ---
$V_{\text{synt,max}}$ | $25 \text{ nM/ms}$ | Maximal velocity at which newly synthesize DA is produce
$K_{\text{synt}}$ | $3\ 500 \text{ nM}$ | Calcium sensitivity
$K_{\text{TYR}}$ | $46\ 000 \text{ nM}$ | Concentration at half maximal velocity
$[\text{TYR}]$ | $126\ 000 \text{ nM}$ | Intracellular tyrosine concentration
$K_{i(cda)}$ | $110\ 000 \text{ nM}$ | Inhibition parameter
$K_{i(eda)}$ | $46\ 000 \text{ nM}$ | Inhibition parameter
$V_{\text{cda,max}}$ | $133.33 \text{ nM/ms}$ | Maximal velocity for VMAT
$K_{\text{CDA}}$ | $23\ 800 \text{ nM}$ | Dopamine concentration at half maximal velocity
$k_{\text{mao}}$ | $0.00016 \text{ ms}^{-1}$ | Intracellular degradation rate
$[\text{DA}]_{v0}$ | $500\ 000\ 000 \text{ nM}$ | Initial concentration of vesicular DA
$[\text{DA}]_{vT}$ | $10\ 000 \text{ nM}$ | Sensitivity to vesicular DA concentrations
$[\text{DA}]_{R_a}$ | $50 \text{ nM}$ | High affinity state for DA binding respect to receptors
$[\text{DA}]_{R_s}$ | $10\ 000 \text{ nM}$ | Binding sensitivity

Table 4.2: Parameter values for the intracellular DA compartment described in Section 4.2 (Best et al., 2009; Bongiovanni et al., 2006; Justice et al., 1988; Kawagoe et al., 1992; Liu and Edwards, 1997; Morgenroth et al., 1976; Near, 1986; Porenta and Riederer, 1982; Royo et al., 2005; Volz et al., 2006; Wimalasena and Wimalasena, 2004). The parameter values for $V_{\text{synt,max}}$, $K_{\text{synt}}$, $[\text{DA}]_{R_s}$ and $k_{\text{mao}}$ were chosen to reflect experimental data.

Hyland et al., 2002; Ross, 1991; Schmitz et al., 2003; Schultz, 2007). Moreover, as seen in Figures 4.12 and 4.13, the three compartment model confirms the sensitivity of extracellular DA concentration to spike frequency while showing that an increase in the duration of the stimulation leads to saturation.

As seen in the left panel of Figure 4.13, our three compartment model predicts that after a 20 seconds simulation of regularly spaced action potentials at 14 Hz, background extracellular DA concentration increases into the range of 50 – 173 nM, a result that overlaps with the 150 – 400 nM range suggested by Gonon (1988). Moreover, the simulations generated by equations 4.1, 4.14, and 4.15 agree with Gonon (1988) and Wightman and Zimmerman (1990), since simulations at various firing frequencies gen-
Figure 4.11: Simulation results showing oscillations in the dopaminergic terminal upon arrival of the action potentials described in the left panel of Figure 4.2 (5.1 Hz). Here the left panel shows that the average cytosolic DA concentration in the terminal bouton is around 1048.7 nM per cycle with a peak at 1244 nM upon the arrival of an action potential. The right panel estimates that once extracellular DA approaches a limit cycle, the amount of DA release per pulse is 65.969 nM of which 62.814 (95.2%) is recycled back into the terminal bouton by DAT. Parameters as in Tables 4.1 and 4.2.

Figure 4.12: Simulation results showing oscillations in the dopaminergic terminal upon arrival of the membrane potential described in the right panel of Figure 4.2 (15.9 Hz). Here the left panel shows the average cytosolic DA concentration in the terminal bouton is approximately 4675.7 nM with a peak at 4682 nM. The right panel shows that bursting behavior increases extracellular DA concentration into the range of 253.1 – 398.8 nM which is in the 150 – 400 nM range suggested by Schultz (1998). Moreover, once extracellular DA approaches a limit cycle, the amount of DA release per pulse is 529.572 nM of which 347.397 (65.6%) is recycled back into the terminal bouton by DAT. Parameters as in Tables 4.1 and 4.2.
erate a DA peak that grows exponentially as shown in the right panel of Figure 4.13.

Figure 4.13: Left panel shows model results for the experiment described in Figure 4.4 which agrees with Wightman and Zimmerman (1990). Note that extracellular DA concentration reaches saturation. Right panel shows that as a function of spike frequency, extracellular DA grows exponentially as proposed by both Gonon (1988) and Wightman and Zimmerman (1990). Parameters as in Tables 4.1 and 4.2.

**Basal concentrations and fluxes**

A hypothetical condition, often referred to as a *steady-state*, is defined as the condition of equilibrium between the rates of formation and clearance in a particular compartment. This term has been loosely used by others studies such as Best et al. (2009), since the concentration of any substance in the brain can fluctuate with time. As such, in order to avoid confusion with mathematical terminology, we use the term *basal concentration* to define the equilibrium between the rates of formation and clearance in a particular compartment.

Assuming a fixed basal concentration of 126 000 nM for TYR (Best et al., 2009) and the instantaneous transformation of L-DOPA to DA, the model shows a linear accumulation of synthesized DA with respect to time, consistent with experimental observations (Cumming, 2009). The model shows an average basal concentration of 1048.7 nM for the cytosolic DA compartment which is consistent with the experimental literature (Cumming, 2009; Liu and Edwards, 1997). We are able to see that at a firing
rate of $5.1\ Hz$ (Figure 4.2), the flux balance for the basal concentration of the cytosolic DA compartment shows that in one hour, approximately $19.607\ mM$ of cytosolic DA is manufactured from synthesized DA and $1.119\ mM$ is recycled back into the cytosol from the extracellular DA compartment. Likewise, $20.127\ mM$ of cytosolic DA is taken up into the vesicles by the vesicular monoamine transporter (VMAT) and $0.601\ mM$ of cytosolic DA is catabolized in the cytosol by MAO.

As mentioned in experimental studies (Ben-Jonathan and Hnasko, 2001; De la Fuente-Fernández et al., 2004; Elsworth and Roth, 2004), the largest portion of cellular DA is in the vesicles. In our model the basal concentration of vesicular DA is within the $0.5 - 0.6\ M$ range. The model predicts that in one hour $1.175\ mM$ of vesicular DA is release into the extracellular DA compartment, most of which, $95.2\%$, is put back into the cytosol. Lastly, the model predicts that after one hour, $0.048\ mM$ of released DA either diffuses away or is catabolized by COMT.

4.4 Overview

The model presented in this chapter is similar in spirit to previously published models, with the advantage that this new approach has the potential to examine the interactions among firing rate, synthesis, and release and the effects of release modulating autoreceptors on the release rate. This interaction is of extreme importance since experimental studies suggest that blocking the firing of substantia nigra-ventral tegmental area of DA neurons leads to an increase in synthesis and release in the target areas of the nigrostriatal and mesolimbic pathway but not in the target area of the mesocortical pathway (Roth, 2004).
Chapter 5

MECHANISMS OF INHIBITION OF THE DOPAMINE UPTAKE CARRIER

The model constructed in Chapter 4 is unique in the sense that to our knowledge, it is the only model capable of reproducing many dynamic features of cytosolic, vesicular, and extracellular DA concentrations while being driven by membrane potentials at the millisecond time scale. As seen at the end of Chapter 4, we selected the source and loss processes of the cytosolic and extracellular DA compartment as primary targets for examination because experimental data are available for comparison. Here we conduct simulations that examine the model responses to changes in parameters and some interaction functions. In particular, these computational studies focus on alterations in mechanisms known to be involved in altered responses due to the application of psychostimulants such as cocaine (COC). The goal is to make predictions about how functional changes in the presynaptic mechanisms due to cocaine modulate exocytosis.

As mentioned in Chapter 1, exocytosis is a presynaptic event that is modulated by a number of processes that are collectively termed “turnover.” See Figure 1.1 for more details.

It is known that psychostimulants promote increases in extracellular DA concentrations via multiple mechanisms. Here we focus on cocaine, which is known to inhibit the activity of DAT (Brown et al., 2001a) and slow the clearance rate of released DA (Greco and Garris, 2003). Other experimental studies find that cocaine treatment and the application of D2 receptor agonists increase the uptake of DA into vesicles (i.e. VMAT-2 activity) (Schmitz et al., 2003). Both of these mechanisms potentially could cause a change in cytosolic DA concentration. Thus any pharmacological or physiological treatment that causes changes in DAT activity most likely would cause changes in the magnitude of released DA and the effective relaxation time of the removal of extracellular DA. We explore how inhibition due to COC can cause alterations in the extracellular DA concentration levels and examine the dynamics of concentrations in
the cytoplasmic DA compartment such as those seen in Figure 4.6 of John and Jones (2007).

5.1 Regulatory mechanisms relevant to the functioning of the DA system

According to Heien and Wightman (2006), MAO inhibition increases the amount of DA available for release while COMT inhibition does not cause a change in the dynamics of DA. To compare our model with these findings, we run simulations showing the effects of intracellular and extracellular degradation of DA by varying the corresponding parameters by factors ranging from $10^{-2}$ to $10^{2}$. Our results agree with these experimental findings since significant decreases in $k_{mao}$ results in slight increases in cytosolic DA and similar decreases in $k_{comt}$ cause essentially no change in both cytosolic and extracellular DA. Numerical results for increases in these parameters, such as those that might be seen in pharmacological studies, suggest that the concentration of cytosolic DA is somewhat sensitive to large increases in $k_{mao}$ but these changes do not dramatically affect the concentration of DA in the extracellular compartment; however, large increases in $k_{comt}$ will affect the extracellular DA concentration.

We also examine the sensitivity to parameters involved in the uptake of DA from the extracellular compartment to the cytosol, $V_{eda,max}$ and $K_{EDA}$. These results are shown in Figures 5.3 and 5.4 and will be considered in the context of the work that follows.

5.2 Kinetic analysis of transporters

In order to examine the possible effects of cocaine on the mechanisms underlying the turnover process, we must consider the effects on $J_{DAT}$ and $J_{VMAT}$. As described in sections 4.1 and 4.2, the construction of $J_{DAT}$ and $J_{VMAT}$ obey carrier-mediated transport kinetics. See Figure 5.5 for an overview of this process.

Under the assumptions described in section 4.1, the reaction found in Figure 5.5 is given by
Figure 5.1: Simulation results showing the effects of the intracellular degradation rate ($k_{mao}$) on the concentration of cytosolic and extracellular DA. We vary $k_{mao}$ by a factor $\alpha$ raging from $10^{-2}$ to $10^{2}$ and plot the percentage change in DA for each value of $\alpha$. Parameters as in Tables 4.1 and 4.2.
Figure 5.2: Simulation results showing the effects of the intracellular degradation rate ($k_{comt}$) on the concentration of cytosolic and extracellular DA. We vary $k_{comt}$ by a factor $\alpha$ ranging from $10^{-2}$ to $10^2$ and plot the percentage change in DA for each value of $\alpha$. Parameters as in Tables 4.1 and 4.2.
Figure 5.3: Simulation results showing the effects of the maximal velocity rate of the DAT transporter ($V_{eda,max}$) on the concentration of cytosolic and extracellular DA. We vary $V_{eda,max}$ by a factor $\alpha$ ranging from 0 to 2 and plot the percentage change in DA versus the percentage change in the parameter. Other parameters as in Tables 4.1 and 4.2.
Figure 5.4: Simulation results showing the effects of the concentration of extracellular DA at the half maximal velocity ($K_{EDA}$) on the concentration of cytosolic and extracellular DA. We vary $K_{EDA}$ within the parameter range $30 - 8000 \text{ nM}$ as suggested by Viggiano et al. (2004) and plot the percentage change in DA for each parameter value. Other parameters as in Tables 4.1 and 4.2.
Figure 5.5: Scheme for DA binding and transport movement. The reaction path proceeding from left to right depicts the binding sequence of DA at the outward facing form of a transporter, $T$, which occurs in two steps. DA and $T$ first combine to create the $DA - T$ transport complex; i.e., the DA is bound to the transporter. This binding is believed to be very fast and reversible with association and dissociation rate constants $k_{ass}$ and $k_{diss}$, respectively. In a second step, chemical processes are initiated where the catalytic rate constant or turnover number, $k_{cat}$, encompasses all of the chemical processes associated with the movement of DA into the membrane. In studies of transporters, experiments generally measure the initial velocity of the transport of DA as a function of DA concentration and fit the result to equation (5.1) (Fersht, 1985; Meiergerd and Schenk, 1994b; Schenk et al., 2005).

$$DA + T \xrightleftharpoons[k_{diss}]{k_{ass}} DA - T \xrightarrow{k_{cat}} T + DA^*$$

This equation contains two parameters, the maximal velocity, $V_{max}$, and the concentration of DA at half maximal velocity, $K_m$. In the case of DAT, we denote the maximal velocity as $V_{eda, max}$, which is given by $V_{eda, max} = k_{cat} \cdot [DAT]$ where $k_{cat}$ represents the catalytic rate constant or turnover number and $[DAT]$ represents the density of the DA transporter site (McElvain and Schenk, 1992; Meiergerd and Schenk, 1994b; Schenk et al., 2005; Volz et al., 2006). In this case we denote the concentration of extracellular DA at half maximal velocity as $K_{EDA} = \frac{k_{diss} + k_{cat}}{k_{ass}} = K_{DA} + \frac{k_{cat}}{k_{ass}}$, where $K_{DA}$ is the dissociation constant of the $DAT - DA_e$ complex (Fersht, 1985). $K_{EDA}$ depends upon the relative magnitude of $k_{diss}$ and $k_{cat}$. If $k_{cat} << k_{diss}$, then $K_{EDA} = K_{DA}$ is the equilibrium constant for the dissociation of DA from DAT. If $k_{cat} >> k_{diss}$ then $K_{EDA} = \frac{k_{cat}}{k_{ass}}$. In this form, the association rate $k_{ass}$ can be approximated by $\frac{k_{cat}}{K_{EDA}}$. Since the catalytic constant, $k_{cat}$, is present in the values of $V_{eda, ma}$ and $K_{EDA}$, if $V_{eda, max}$ changes with no associated change in $K_{EDA}$, then one may assume that the density of DAT has changed. On the other hand, if $V_{eda, max}$ and $K_{EDA}$ change simultaneously, then it may be assumed that $k_{cat}$ may have changed. Finally, if $K_{EDA}$ changes without a change in $V_{eda, max}$, then the binding of extracellular DA at DAT can be assumed to have changed (Schenk et al., 68).
2005). See Figure 5.6 for a representation of the reaction found in Figure 5.5 with DAT as the transporter and details regarding $K_{EDA}$.

5.3 Cocaine

Cocaine is an important psychostimulant that exerts its addictive and psychomotor effects by elevating and depleting extracellular and cytosolic DA concentration, respectively, in part due to its ability to inhibit DAT (Brown et al., 2001b) and activate DA receptors (Brown et al., 2001a). The experimental literature gives conflicting views of the mechanisms of uptake inhibition including: (1) competitive inhibition (Huang et al., 2009; Krueger, 1990; Sotnikova et al., 2005) by altering $K_{EDA}$ (Cornish-Bowden, 2004; Greco and Garris, 2003), (2) uncompetitive inhibition (McElvain and Schenk, 1992; Meiergerd and Schenk, 1994b) by altering both $K_{EDA}$ and $V_{eda,max}$ (Cornish-Bowden, 2004; Greco and Garris, 2003) and (3) noncompetitive inhibitory kinetics (Chen and Justice, 1998; Greco and Garris, 2003; Missale et al., 1985) by altering $V_{eda,max}$ (Cornish-Bowden, 2004; Greco and Garris, 2003). According to Meiergerd et al. (1994) the apparent mechanisms of inhibition in a transport experiment may vary depending on the experimental conditions.

**Inhibition of the extracellular DA transporter by cocaine**

Theoretically, different inhibition patterns can occur if COC and DA bind to DAT at different sites (Meiergerd and Schenk, 1994a). See Figure 5.7 for details. There are three potential binding patterns for COC and DA to bind to DAT: (1) COC binds to DAT to give rise to the $DAT/COC$ complex with dissociation constant $K_{i(COC)}$, (2) COC binds to the $DAT − DA_e$ complex to give rise to the $DAT − DA_e/COC$ complex with dissociation constant $K_{i(EDA−COC)}$, and (3) COC binds to a site that is distinct from the extracellular DA binding site; therefore, it can bind to both the free $DAT$ and the $DAT − DA_e$ complex (Huang et al., 2009). See Figure 5.7 for the reaction scheme and Figure 5.8 for a schematic.
Figure 5.6: Cartoon model of the transport kinetics reaction shown in Figure 5.5 with DAT as the transporter. Extracellular DA binds to DAT, and DAT catalyzes the transport of DA across the neuronal membrane by undergoing conformational changes and releasing DA into the cytosol (Meiergerd and Schenk, 1994b; Schenk, 2002). See the text for additional details.

In accordance with Cornish-Bowden (2004), since \( \text{DA}_e \) can be released in a step that generates complexes other than the DAT – \( \text{DA}_e \) complex, COC acts as a mixed inhibitor with maximal velocity, \( V^{\text{COC}}_{\text{eda,max}} \), and half maximal constant, \( K^{\text{COC}}_{\text{EDA}} \), defined as

\[
V^{\text{COC}}_{\text{eda,max}} = \frac{V_{\text{eda,max}}}{1 + \frac{[\text{COC}]}{K_i(\text{EDA} \rightleftharpoons \text{COC})}} \quad (5.2)
\]

and

\[
K^{\text{COC}}_{\text{EDA}} = \frac{K_{\text{EDA}} \left(1 + \frac{[\text{COC}]}{K_i(\text{COC})}\right)}{1 + \frac{[\text{COC}]}{K_i(\text{EDA} \rightleftharpoons \text{COC})}} \quad (5.3)
\]

and the unidirectional flux of DA in the presence of COC, \( J_{\text{DAT:COC}} \), is given by
Figure 5.7: Illustration for the mechanisms that produce mixed inhibition on DAT in the presence of COC. The binding site for DAT with DA and COC illustrates competitive inhibition. A competitive relationship between COC and $Na^+$ along with an allosteric relationship between DA and $Na^+$ leads to the expectation of a non-competitive or uncompetitive relationship between DA and COC, suggesting separate binding sites for the binding of DAT (Meiergerd et al., 1994).

$$J_{DAT:COC} = \frac{V_{eda,max} \cdot [DA]_e}{[DA]_e \left(1 + \frac{[COC]}{K_{i(EDA-COC)}}\right) + K_{EDA} \left(1 + \frac{[COC]}{K_{i(COC)}}\right)}.$$ (5.4)

Equation (5.4) represents the general case for all types of product inhibition with the introduction of three new parameters, $[COC]$, $K_{i(COC)}$, and $K_{i(EDA-COC)}$. As $K_{i(EDA-COC)} \rightarrow \infty$, this simplifies to the form of competitive inhibition (equation 5.6). As $K_{i(COC)} \rightarrow \infty$, the uncompetitive inhibition form (equation 5.8) is obtained, and the form for “purely” non-competitive inhibition will be obtained when $K_{i(EDA-COC)} = K_{i(COC)}$ (equation 5.11). In the presence of an inhibitor, the percent activity is commonly defined as $J_{DAT:COC}/J_{DAT}$ (Wu et al., 2003). In similar fashion, in order to investigate potential mechanisms that overcome the activity of COC, we examine the ratio of $J_{DAT}$ (equation 4.11) to $J_{DAT:COC}$ (equation 5.4) given by
\[
\frac{J_{DAT}}{J_{DAT::COC}} = 1 + \frac{[COC]}{K_{i(COC)}} \cdot \left( \frac{1}{K_{EDA}} \cdot \left( \frac{[DA]_e}{K_{EDA}} + \frac{1}{1 + \frac{[DA]_e}{K_{EDA}}} \right) \right)
\]

since any mechanism that moves equation (5.5) closer to one, reduces the impact of inhibition. With this in mind, we examine the other three possible forms of inhibition.

1. **Competitive Inhibition.** If COC binds to DAT and prevents DA binding and vice versa, COC and DA may be competing with each other to bind with DAT. Competitive inhibition is a limiting behavior case of mixed inhibition (equation 5.4) in which \(K_{i(EDA::COC)}\) approaches infinity (Cornish-Bowden, 2004). Thus, if we think of COC
as a competitive inhibitor, it would follow the kinetics scheme found in equation (1) of Brandt et al. (1987) and equation (5.4) may be written as

\[
J_{\text{DAT:COC}} = \frac{V_{\text{eda,max}} \cdot [\text{DA}]_e}{[\text{DA}]_e + K_{\text{EDA}} \left(1 + \frac{[\text{COC}]}{K_{i(COC)}}\right)}.
\] (5.6)

The presence of COC as a competitive inhibitor will increase \(K_{\text{EDA}}\) by a factor of \(1 + \frac{[\text{COC}]}{K_{i(COC)}}\). Equation (5.6) indicates that \(J_{\text{DAT:COC}}\) is dependent on the concentration of COC and extracellular DA. In this situation, the contribution of \(\text{DAT} - \text{DA}_e/\text{COC}\) binding is negligible in comparison with that of \(\text{DAT}/\text{COC}\) binding as proposed by Huang et al. (2009). Therefore, we conclude that \(\text{DAT}\) binds to either COC or extracellular DA. An analysis of equation (5.6) shows that \(J_{\text{DAT:COC}}\) is a decreasing function of COC, which tends to zero when \([\text{COC}] >> K_{i(COC)}\). In this case, DAT will only bind to COC, producing inhibition on the uptake of extracellular DA. Hence, the clearance of extracellular DA will depend solely on \(J_{\text{eda}}^o\) (equation 4.12), causing an increase in the relaxation time of extracellular DA. See the top panel of Figure 5.9. In addition, as \(K_{i(\text{EDA-COC})} \to \infty\), equation (5.5) reduces to

\[
\frac{J_{\text{DAT}}}{J_{\text{DAT:COC}}} = 1 + \frac{[\text{COC}]}{K_{i(COC)}} = 1 + \frac{K_m \cdot [\text{COC}]}{[\text{DA}]_e \cdot K_{i(COC)} + K_m \cdot K_{i(COC)}},
\] (5.7)

indicating that to overcome competitive inhibition, the concentration of extracellular DA needs to increase relative to that of COC as seen in the bottom panel of Figure 5.9. In other words, competitive inhibition is only effective when \([\text{DA}]_e << [\text{COC}]\) as suggested by Meiergerd and Schenk (1994a).

2. Uncompetitive Inhibition. The other limiting case of mixed inhibition in which \(K_{i(COC)}\) approaches infinity is known as uncompetitive inhibition (Cornish-Bowden, 73}
Figure 5.9: Velocity of the uptake of DA as a function of extracellular DA and competitive inhibition by cocaine with $K_i(COC) = 58 \text{nM}$ (John and Jones, 2007). The top panel shows the extracellular DA concentration as a function of the amount of COC under competitive inhibition. As the amount of COC gets larger, equation (5.6) for $J_{DAT,COC}$ tends to zero, producing a total blockage of the $DAT - DA_e$ complex formation which in turns increases the relaxation time of extracellular DA. The bottom panel shows the velocity of the uptake of extracellular DA under the same inhibition. An analysis of equation (5.7) reports that increases in extracellular DA will overcome competitive inhibition; that is, inhibition is only effective when $[DA]_e << [COC]$ as predicted by Meiergerd and Schenk (1994a). Parameters as in Tables 4.1 and 4.2.
If we think of COC as an uncompetitive inhibitor, we obtain the kinetic scheme found in equation (12) of Brandt et al. (1987) and equation (5.4) may be written as

\[ J_{\text{DAT:COC}} = \frac{V_{\text{eda,max}} \cdot [DA]_e}{[DA]_e \left( 1 + \frac{[COC]}{K_{i(EDA-COC)}} \right) + K_{EDA}}. \] (5.8)

The presence of COC as an uncompetitive inhibitor will decrease the term \( k_{\text{cat}} \) in \( V_{\text{eda,max}} \) and \( K_{EDA} \) by a factor of \( 1 + \frac{[COC]}{K_{i(EDA-COC)}} \). Equation (5.8) indicates that \( J_{\text{DAT:COC}} \) is dependent on the concentration of COC and extracellular DA. In this situation the contribution of DAT/COC binding is negligible in comparison with that corresponding to DAT – DA\(_e\)/COC complex binding (Fersht, 1985). Moreover, the term \( K_{EDA} = \frac{k_{\text{dis}}}{k_{\text{ass}}} + \frac{k_{\text{cat}}}{k_{\text{ass}}} \) in the absence of COC can now be approximated by \( K_{EDA} \approx K_{DA} \) in the presence of COC, suggesting that DAT is no longer capable of transporting extracellular DA across the neural membrane. See Figure 5.7 for details. Therefore, since DAT/COC binding is negligible and DAT can no longer transport extracellular DA across the neuronal membrane, we conclude that the DAT – DA\(_e\) complex binds only to COC (Cornish-Bowden, 2004; Fersht, 1985). An analysis of equation (5.8) shows that \( J_{\text{DAT:COC}} \) is a decreasing function of COC, which tends to zero when \( [COC] \gg K_{i(EDA-COC)} \). In this case, even though there is inhibition of the uptake of extracellular DA, we can deduce that COC cannot inhibit the DA uptake process until DA is bound to DAT, suggesting that unlike competitive inhibition, there will be no change in the relaxation time of extracellular DA as demonstrated in Figure 5.10. In addition, as \( K_{i(COC)} \to \infty \), equation (5.5) reduces to

\[ \frac{J_{\text{DAT}}}{J_{\text{DAT:COC}}} = 1 + \frac{\frac{[COC]}{K_{i(EDA-COC)}} \cdot [DA]_e}{1 + \frac{[DA]_e}{K_{EDA}}} = \frac{K_{EDA} + [DA]_e \cdot \left( 1 + \frac{[COC]}{K_{i(COC)}} \right)}{K_{EDA} + [DA]_e}. \] (5.9)
If conditions are such that \([DA]_e >> K_{EDA}\) then the above equation can be approximated by

\[
\frac{J_{DAT}}{J_{DAT:COC}} = 1 + \frac{[COC]}{K_{i(EDA-COC)}}
\]  \hspace{1cm} (5.10)

indicating that unlike competitive inhibition, uncompetitive inhibition can not be reversed by increasing the concentration of extracellular DA. See the bottom panel of Figure 5.10. In other words uncompetitive inhibition is effective when the extracellular DA concentration is high as suggested by Meiergerd and Schenk (1994a).

3. Non-competitive Inhibition. The last form of inhibition to consider in the presence of COC is non-competitive inhibition. According to Cornish-Bowden (2004), in non-competitive inhibition the dissociation constants \(K_{i(COC)}\) and \(K_{i(EDA-COC)}\) must be equal. Under such a condition, equation (5.4) reduces to

\[
J_{DAT:COC} = \frac{V_{eda,\text{max}}}{\left(1 + \frac{[COC]}{K_{i(EDA-COC)}}\right)} \cdot \frac{[DA]_e}{[DA]_e + K_{EDA}}
\]

\[
= \frac{[DA]_e}{\left(1 + \frac{COC}{K_{i(COC)}}\right) \cdot ([DA]_e + K_{EDA})}
\]  \hspace{1cm} (5.11)

indicating that cocaine does not bind to the DA site but binds to the \(DAT-DA\) site and acts by reducing its turnover rate of translocation by a factor of \(1 + \frac{COC}{K_{i(COC)}}\). The reaction scheme found in Figure 5.7 details the mechanism for COC as a non-competitive inhibitor if we assume that \(K_{i(COC)} = K_{i(EDA-COC)}\). Here the binding of COC and DA are completely independent, and the binding of COC results in the total inhibition of the catalytic step (Cornish-Bowden, 2004). Equation (5.11) indicates that \(J_{DAT-COC}\)
Figure 5.10: Velocity of the uptake of DA as a function of extracellular DA and uncompetitive inhibition by cocaine with $K_{i(E_{DA-COC})} = 1050\text{nM}$ (Meiergerd and Schenk, 1994b). The top panel shows the extracellular DA concentration as a function of the amount of COC under uncompetitive inhibition. As the amount of COC gets larger, equation (5.8) for $J_{DAT\cdot COC}$ tends to zero, producing a total blockage of the $DAT\cdot DA_e$ complex formation which unlike competitive inhibition does not influence the relaxation time of extracellular DA. The bottom panel shows the velocity of the uptake of extracellular DA under the same inhibition. An analysis of equation (5.9) reports that unlike competitive inhibition, increases in extracellular DA will have no effect on overcoming uncompetitive inhibition which means that inhibition is effective with higher values of extracellular DA as predicted by Meiergerd and Schenk (1994a). Parameters as in Tables 4.1 and 4.2.
is independent of $DA_e$ and the relaxation time depends on the affinity of the inhibition constant $K_{i(COC)}$. See Figures 5.11 - 5.13. An analysis of

$$\frac{J_{DAT}}{J_{DAT:COC}} = 1 + \frac{[COC]}{K_{i(COC)}}$$

(5.12)

confirms that non-competitive inhibition is independent of extracellular DA and depends heavily upon the relative magnitude of $K_{i(COC)}$ and COC. Figures 5.11 - 5.13 illustrate the behavior of $J_{DAT:COC}$ for three different values of $K_{i(COC)}$ found in the literature. We find that as $K_{i(COC)}$ becomes relatively close to the concentration of COC, the relaxation time of extracellular DA is unaffected, suggesting that the ratio of $\frac{[COC]}{K_{i(COC)}}$ is of significance when comparing results found in the literature.

*Simulation results for the kinetics of DA-DAT interactions and possible mechanisms of inhibition of DA transport by cocaine*

Within the literature there are many publications that favor a particular form of inhibition over the other and provide justifications for that form. For example, Westley and Westley (1996) states that competition among extracellular DA and COC may not be an appropriate basis for the design of potential therapeutic agents and indicate that uncompetitive inhibition is a far more efficient method. Likewise, Cornish-Bowden (2004) states that non-competitive inhibition is rare and usually a re-evaluation of the original data indicates mixed inhibition. Moreover, Buxser and Vroegop (2005) criticize the use of uncompetitive inhibition as a result of inaccurate data analysis. Thus to compare the four inhibitory methods, we examine the percent inhibition (Wu et al., 2003) defined by
Figure 5.11: Velocity of the uptake of DA as a function of extracellular DA and non-competitive inhibition by cocaine with $K_i(COC) = 58 \, nM$ (John and Jones, 2007). The top panel shows the extracellular DA concentration as a function of the amount of COC under non-competitive inhibition. As the amount of COC gets larger, equation (5.11) for $J_{DAT,COC}$ displays the same dynamics as those observed for competitive inhibition. The bottom panel shows the velocity of the uptake of extracellular DA under the same inhibition. An analysis of equation (5.12) reports that non-competitive inhibition is independent of extracellular DA as predicted by Meiergerd and Schenk (1994a). Parameters as in Tables 4.1 and 4.2.
Figure 5.12: Velocity of the uptake of DA as a function of extracellular DA and non-competitive inhibition by cocaine with $K_{i(COC)} = 270 \text{nM}$ (Povlock and Schenk, 1997; Giros et al., 1994). The top panel shows the extracellular DA concentration as a function of the amount of COC under non-competitive inhibition. Simulations suggest that, unlike the previous case, COC has no effect on the relaxation time at low concentrations. The bottom panel shows the velocity of the uptake of extracellular DA under the same inhibition. Parameters as in Tables 4.1 and 4.2.
Figure 5.13: Velocity of the uptake of DA as a function of extracellular DA and non-competitive inhibition by cocaine with \( K_{i,EDA-COC} = 1050 \text{nM} \) (Meiergerd and Schenk, 1994b). The top panel shows the extracellular DA concentration as a function of the amount of COC under non-competitive inhibition. As the amount of COC gets larger, equation (5.11) for \( J_{DAT:COC} \) tends to zero, producing a total blockage of the \( DAT-DA_e \) complex formation. It is worth noticing that when examined together Figures 5.11 - 5.13 indicate that under non-competitive inhibition if \( COC \leq 6K_{i(COC)} \) then the complex formation will not interfere with the relaxation time. The bottom panel shows the velocity of the uptake of extracellular DA under the same inhibition. An analysis of equation (5.12) reports that \( J_{DAT:COC} \) displays the same dynamics as those observed for uncompetitive inhibition. Parameters as in Tables 4.1 and 4.2.
\[ \% \text{Inhibition} = 100 \cdot \left( 1 - \frac{J_{\text{DAT:COC}}}{J_{\text{DAT}}} \right) \]

\[ = 100 \cdot \frac{[\text{COC}]}{K_{i(COC)} \cdot \left( 1 + \frac{[DA]_e}{K_{EDA}} \right)^2 + [\text{COC}]} \quad (5.13) \]

where \( J_{\text{DAT:COC}} \) and \( J_{\text{DAT}} \) are the velocity of extracellular DA in the presence and absence of COC at a fixed concentration. Figure 5.14 shows the qualitative difference among the inhibitory methods in the presence of 1000 nM of COC. The numerical results show that the quantitative differences among the distinct mechanisms are all within some margin of error of the general form found in the mixed inhibition mechanism (equation 5.13). Thus in order to minimize the size and complexity of the equations that follow, we focus on the general form for the mixed inhibitory mechanism.

**Quantitative comparisons of cocaine affinity**

According to Meiergerd and Schenk (1994a), apparent patterns of inhibition of transport vary with the type of transport experiment being conducted. The numerical experiments presented above examine the importance of the values of \( K_{EDA}, V_{eda,\max}, \)

\( K_{i(COC)} \) and \( K_{i(EDA-COC)} \) and their interpretation within the context of the experiments. Typically, experiment results report these values after fitting the data to the Lineweaver-Burk plot (equation 5.14) or the Eadie-Hofstee plot (equation 5.15)

\[ \frac{1}{J_{\text{DAT:COC}}} = \left( \frac{K_{EDA}}{V_{eda,\max}} \right) \cdot \left( \frac{1}{[DA]_e} \right) + \frac{1}{V_{eda,\max}} \quad (5.14) \]

\[ J_{\text{DAT:COC}} = V_{eda,\max} - K_{EDA} \cdot \left( \frac{J_{\text{DAT:COC}}}{[DA]_e} \right). \quad (5.15) \]
Figure 5.14: Illustration of the different functional consequences of competitive, uncompetitive, non-competitive and mixed inhibition of the transport of extracellular DA for 1000 nM of COC. The percent inhibition values for each mechanism were obtained via the modifications of the general equation for mixed inhibition (equation 5.13). The $J_{DAT:COC}$ for each mechanism is described in the corresponding sections of the text. The simulation results present the same qualitative behavior as the conceptual approach in Figure 3 of Meiergerd and Schenk (1994a).

As seen in Figure 5.15, these equations behave differently for various concentrations of COC. Typically, the x and y intercepts, as well as the slope of the lines in either plot, found in Table 5.1, are used by experimentalists to estimate the values of $K_{COC}^{EDA}$ and $V_{max,eda}^{COC}$. See Figure 5.16. Typically, their interpretation of $K_i$ will depend on the presumed mechanisms for inhibition. In our numerical studies using mixed inhibition, both of these quantities depend on the numerical values of $K_{EDA}$, $K_{i(COC)}$, $K_{i(EDA-COC)}$, and the concentration of COC. According to Schenk (2002), the COC inhibition constant $K_i$ ought to be close in value to $K_{EDA}$. Various experimental studies have found the ranges of $K_{i(COC)}$ and $K_{i(EDA-COC)}$ to be around $58 \text{ -- } 1220$ nM and $850 \text{ -- } 1050$ nM.
respectively. Hence, if it is the case that values for a particular quantity vary widely as is the case for $K_i(COC)$ and $K_{EDA}$, then the reported experimental results may not be accurate. Figures 5.17 - 5.20 show numerical solutions for the Lineweaver-Burk plot and the Eadie-Hofstee plot based on different values of $K_i(COC)$ ranging from $58 \text{nM}$ to $10000 \text{nM}$. These numerical results clearly show that incorrect values of $K_i(COC)$ can lead to faulty interpretation of data and behavioral effects, such as the inhibitory effects of COC on the uptake of DA, $J_{COC:DAT}$. In these simulations, $K_{EDA} = 30 \text{nM}$ and $K_i(EDA-COC) = 1050 \text{nM}$.

<table>
<thead>
<tr>
<th>Plot</th>
<th>x-intercept</th>
<th>y-intercept</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lineweaver-Burk</td>
<td>$\frac{1 + [\text{COC}]<em>{i(EDA-COC)}}{V</em>{eda,\text{max}}}$</td>
<td>$\frac{(1 + [\text{COC}]<em>{i(EDA-COC)}}{K</em>{ED} \cdot (1 + [\text{COC}]_{i(EDA-COC)}}$</td>
<td>$\frac{1 + [\text{COC}]<em>{i(EDA-COC)}}{V</em>{eda,\text{max}}}$</td>
</tr>
<tr>
<td>Eadie-Hofstee</td>
<td>$\frac{V_{eda,\text{max}}}{K_{EDA} \cdot (1 + [\text{COC}]_{i(EDA-COC)}}$</td>
<td>$\frac{V_{eda,\text{max}}}{(1 + [\text{COC}]_{i(EDA-COC)}}$</td>
<td>$-K_{EDA} \cdot \left(1 + \frac{[\text{COC}]}{K_{i(COC)}}\right)$</td>
</tr>
</tbody>
</table>

Table 5.1

When examining the values for the x and y-intercepts and slopes for both approaches as described in Table 5.1, one can deduce that regardless of the type of inhibition, all results can be explained through examination and manipulations of the specificity constant, $\frac{k_{cat}}{K_{EDA}}$, and its interplay with the inhibitory factor $1 + \frac{[\text{COC}]}{K_{i(COC)}}$. The application and usefulness of the ratio $\frac{k_{cat}}{K_{EDA}}$ has previously been explored by Koshland (2002) and Eisenthal et al. (2007); hence, we focus on the concentration of COC in simulations that examine the effects of COC concentration for fixed $K_i(COC)$. Numerical results (see Figure 5.21) demonstrate that increments in the concentration of COC have a strong effect on both the relaxation time and the velocity of DAT, thus we further examine the percent inhibition as shown in Figure 5.22. It is this behavior which experimentalists use to determine inhibitory mechanisms. For example, in the case of competitive inhibition, $K_{EDA}$ should increase linearly with respect to COC concentration. For a complete description of each case we recommend comparisons to Table 3.3.
Figure 5.15: Mixed inhibition by cocaine (COC) of DA uptake with \( K_i(\text{COC}) = 58 \text{nM} \). Top panel shows the plot of \( \frac{1}{J_{\text{DAT:COC}}} \) against \( \frac{1}{[\text{DA}]_e} \), commonly known as the Lineweaver-Burk plot. This is the most widely used approach for estimating parameters, even though it is not very accurate for small or large values of \( J_{\text{DAT:COC}} \). The bottom panel shows the plot of \( J_{\text{DAT:COC}} \) against \( J_{\text{DAT:COC}}^{-1}[\text{DA}]_e \), commonly known as the Eadie-Hofstee plot. The presence of \( J_{\text{DAT:COC}} \) on both coordinates means that errors in \( J_{\text{DAT:COC}} \) could potentially change the behavior of the entire system. Results with \( K_{\text{EDA}} = 30 \text{nM} \) and \( K_i(\text{EDA–COC}) = 1050 \text{nM} \) are comparable with those seen in Figure (6) of Krueger (1990) and Figure (3) of Chen and Justice (1998). Parameters as in Tables 4.1 and 4.2.
Figure 5.16: The effects of cocaine (COC) on $K_{COC}^{EDA}$ and $V^{COC}_{max,eda}$. The top panel shows that COC decreases $V^{COC}_{max,eda}$. The bottom panel shows that COC increases $K_{COC}^{EDA}$. A comparison with Figure 5.15 provides useful information about the estimation of the $V_{max}$ and $K_m$ values found in equation 5.1. Our results are comparable with those seen in Figure (3) of Chen and Justice (1998). Parameters as in Tables 4.1 and 4.2.
of Copeland (2005).

Figure 5.17: Mixed inhibition by cocaine (COC) of DA uptake with $K_{i(COC)} = 270 \text{nM}$. In its current form, panel (c) and (d) indicate that our numerical solutions resemble results from experiments where COC is thought to be an uncompetitive inhibitor since both both values, $K_{EDA}$ and $V_{\text{max,eda}}$, are varying. The graphs have the same representation as those found in Figure 5.15 and Figure 5.16. Our results for panel (a) are comparable to Figure (6) of McElvain and Schenk (1992). Parameters as in Tables 4.1 and 4.2.

**Summary of results**

The goal of this dissertation was to integrate previous experimental, theoretical, and computational research in order to develop a model for the turnover process in DA neurons. The model constructed in Chapter 4 consists of differential equations representing the cytosolic, vesicular, and extracellular compartments that describe the dynamics of DA synthesis, storage, release and reuptake while being driven by a previously published model of a mesencephalic DA neuron (Kuznetsov et al., 2006). Numerical results for this model agree with essential results found in the neuroscience literature. For example, experimental data suggest that resting levels of intracellular calcium at release sites are approximately $0.1 \, \mu M$ and can rise to $5 - 10 \, \mu M$ upon arrival of an ac-
Figure 5.18: Mixed inhibition by cocaine (COC) of DA uptake with $K_i(COC) = 790\, nM$. The graphs have the same representation as those found in Figure 5.15 and Figure 5.16. Examining panels (c) and (d) suggest an uncompetitive or non-competitive form depending on whether $K_i(COC)$ is interpreted as varying. Parameters as in Tables 4.1 and 4.2.

After establishing the model’s validity in Chapter 4, the model was used to make predictions about the functional changes in the presynaptic mechanisms due to cocaine. In these studies, we examined the modulation of exocytosis via two distinct
mechanisms: (1) sensitivity to parameters involved in intracellular/extracellular degradation and the reuptake of DA from the extracellular compartment to the cytosol and (2) kinetic analysis of the DA transporter under the presence of COC as an inhibitor. The model’s response for sensitivity analysis due to changes in reuptake parameters (Figures 5.3 and 5.4) is consistent with the results seen in the kinetic analysis of DAT (Figures 5.10 - 5.13) to a very significant degree. Analysis of the mechanisms underlying inhibition shows that either $K_{EDA}$ or $V_{eda,max}$ (or both) vary in the presence of cocaine. According to our sensitivity analysis, decreases in $V_{eda,max}$ lead to substantial increases in extracellular DA as expected with cocaine. This agreement suggests that further sensitivity results such as those in the top panel of Figure 5.3 are reliable, extending our understanding of DA dynamics. Similarly, sensitivity analysis for increases in $K_{eda}$ reveal substantial increases in extracellular DA. In examining numerical results for a mixed inhibition mechanism and comparing to competitive, non-competitive and
uncompetitive inhibition mechanisms, we noted many behavioral similarities for these different types of inhibition that depend on inhibition parameters and levels of cocaine. For example, when the two inhibitory dissociation parameters for mixed inhibition are similar, but not necessarily equal, numerical results which mimic those examined in experimental paradigms are indistinguishable from results that one would obtain with either non-competitive or uncompetitive inhibition. Another example is that for extremely large values of $K_i(\text{COC})$, the qualitative results for mixed inhibition match those expected for non-competitive inhibition. Along these lines, in specific numerical experiments which focus on examining possible inhibition mechanisms for COC as an inhibitor of DAT (Figures 5.15 - 5.22) presented in Chapter 5, we demonstrate qualitatively similar results to many found in the neuroscience literature and provide a different possible interpretation of these results within the context of mixed inhibition. These results provide a possible explanation for the conflicting views of uptake inhibi-
Figure 5.21: Velocity of the uptake of DA as a function of extracellular DA, and mixed inhibition by cocaine with $K_{i(EDA-COC)} = 1050$ and $K_{i(COC)} = 58 \text{nM}$ (John and Jones, 2007). The top panel shows the extracellular DA concentration as a function of the amount of COC under mixed inhibition, which appears similar to the results for competitive inhibition as well as non-competitive inhibition with $K_{i(COC)} = 58$. The bottom panel shows the velocity of the uptake of extracellular DA under the same mixed inhibition, and looks similar to results for uncompetitive as well as non-competitive inhibition with $K_{i(COC)} = 70$. Parameters as in Tables 4.1 and 4.2.
Concerning future work, here DA synthesis is assumed to be a function of $Ca^{2+}$. In further work, one might integrate the model proposed by Kotter and Schirok (1999) with the one presented here in order to provide a more comprehensive study of the dopaminergic system. Further, our numerical results suggest that COC is likely a mixed inhibitor with two similar dissociation constants, which implies that COC competes with $Na^{+}$ for its binding sites. If this is the case, a re-evaluation of equation (4.11) is in order. A more accurate model equation for $J_{DA}$ might incorporate extracellular DA, vesicular DA, and $Na^{+}$ and would be similar in spirit to the one proposed by Schenk (2002).


BIOGRAPHICAL SKETCH

David Tello was born in Lima-Perú and migrated to Chicago, IL at the age of 15. He attended Theodore Roosevelt High School, one of many Chicago Public Schools in the Albany Park district and graduated in 1995. Starting the Fall of 1995, he was fortune to attend the University of Illinois in Chicago (UIC) for his undergraduate studies.

Unaccustomed to hard work, his beginnings at UIC were disappointing. After three semesters at UIC, he was placed on academic probation, facing possible dismissal from the university. By then, he had the fortune to have met the director of the Rafael Cintrón-Ortiz Latino Cultural Center and adjunct Associate Professor of Mathematics, Dr. Rodrigo Carramiñana, who played a life-changing role in his academic and personal life.

Dr. Carramiñana became his instructor in several undergraduate mathematics courses including an independent study in group theory. In the summer of 1999, mainly due to Carramiñana’s kind intervention, he was given the opportunity to participate in his first summer research program despite his poor academic record. Under the guidance of Dr. Carramiñana, he successfully completed the summer program. This academic triumph opened the door to participation in two nationally funded research programs, another summer research opportunity at the University of Iowa, and inspired him to do better academically. Carramiñana and Tello’s interaction went far beyond the scope of the classroom walls, since for over a period of five years, Tello interacted with Dr. Carramiñana on a daily basis at the Latino Cultural Center where Carramiñana was Tello’s supervisor. During this time Dr. Carramiñana’s preferred daily phase for Tello was “estudia huevón porque es la única forma de salir de la pobreza!” Dr. Carramiñana taught Tello how to read a math book, how to rationalize a problem, and how to think before jumping into any solutions. When Tello first met Dr. Carramiñana in 1995, he was a freshman student with no guidance or goals and when he left Carramiñana in July of 2001, Tello was an admitted master student and a NSF Rackham Merit Fellowship holder at the Department of Mathematics of University of Michigan with one goal in mind: “to obtain a Ph.D. in Mathematics.”