

A Graph Theoretic Mathematical Model for Alzheimer's disease

Using a systems biology approach

C. Rose Kyrtos

Institute for Systems Research
University of Maryland
College Park, MD, US
ckyrtsos@hmc.psu.edu

John S. Baras

Institute for Systems Research &
Department of Electrical Engineering
University of Maryland
College Park, MD, US
baras@umd.edu

Abstract— Alzheimer's disease (AD) is currently the most common form of dementia affecting the elderly, and its occurrence rate is only expected to increase over the next several decades. Though there is a vast array of knowledge about individual molecules and genetics involved with the disease, there is no clear understanding of the mechanism of pathogenesis. To help better understand the disease process, a graph theoretic model was developed that studies both the concentration of molecules thought to be involved in pathogenesis ($A\beta$, interleukin-1, tumor necrosis factor alpha, cholesterol, ATP levels), as well as the cell number in a small location in the brain. Particular emphasis was put on the role of the inflammatory process in AD progression. This represents one of the first models that uses graph theory combined with a systems biology approach to study AD.

Keywords— Alzheimer's disease, math model, systems biology, graph theory, inflammation

I. INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia affecting the elderly, and its occurrence rate is expected to increase in coming years as the average age of the US population increases. AD is characterized by the loss of short-term memory, with progressive loss of long-term memory as the disease progresses [19, 31]. Other symptoms include depression, labile mood, loss of voluntary muscle control and cognitive decline. There is currently no known effective treatment to prevent or stop the progression of AD, and the disease often leads to fatal complications.

At the cellular level, AD has been characterized by the presence of beta amyloid ($A\beta$) plaques and neurofibrillary tangles of the tau protein on histological examination [12]. Recent studies have shown that the oligomeric form of $A\beta$ is neurotoxic and can suppress long term potentiation necessary for memory formation [34]. Other studies have drawn attention to the importance of neuroinflammation in the disease process, citing that inflammation may play a significant role in AD initiation and progression [1, 15, 21, 30]. Beta amyloid induces the production of pro-inflammatory cytokines (IL-1, TNF α), which may lead to subsequent

increased production of $A\beta$, thus creating a positive feedback loop that leads to progressively higher levels of $A\beta$ and inflammation [35-37]. The pro-inflammatory environment itself is detrimental to neurons and can lead to neuronal loss and synaptic dysfunction over time. Inflammation is balanced by the generation of IL-6 by microglia, a cytokine that inhibits the secretion of IL-1 and TNF α .

Dysregulation of lipid metabolism has also been implicated in AD [3, 6, 14, 16, 23]. Cholesterol has been identified as one of the key lipids that is dysregulated, though its exact mechanism in the AD process is not well understood. It is believed that high levels of plasma cholesterol and low levels of brain cholesterol correspond to a significantly increased risk for AD. Cholesterol has also been shown to inhibit the production of beta amyloid, though some studies do not agree with this conclusion [9, 16, 24].

ATP levels and mitochondrial dysfunction have also been observed in AD [22, 39-42]. Depletion of ATP leads to a cascade of worsening mitochondrial dysfunction and cellular damage [43]. Decreased ATP initially leads to decreased functioning of several key channels, most notably failure of the Na⁺/K⁺ ATPase (leads to accumulation of intracellular Na⁺, cell swelling and dilation of the ER) and failure of the Ca²⁺ pump (leads to influx of Ca²⁺. Release of Ca²⁺ from intracellular stores (especially the mitochondria) occurs early during the insult, and is maintained by Ca²⁺ influx across the dysfunctioning plasma membrane. If maintained for extended periods of time, ATP depletion will eventually lead to protein misfolding and a significant reduction in protein synthesis. The increased intracellular Ca²⁺ levels leads to activation of the mitochondrial permeability transition pore, activation of endonucleases, proteases, phospholipases and ATPases; and the induction of apoptosis via release of cytochrome c into the cytosol when permeability of the outer membrane of the mitochondria is increased.

Given the complexity of the networks involved in AD, mathematical modeling provides an excellent approach to studying the effects of alterations in one of the many molecules that has been suggested to play a role in AD

pathogenesis. Few models have been developed to date that studies AD from a systems biology perspective. Previously developed models have used differential equations to study how microglia respond to chemotactic signals, as well as studied basic interactions between neurons and other cell types [4,7]. To date, no model has taken a systems approach and studied both the role of cholesterol and inflammation in the pathogenesis of AD, as has been done here.

This paper gives a brief overview of the mathematical model that has been derived. A graph theoretic approach has been used to study the network, with each node representing a chemical species and edges representing a reaction or interaction. Particular focus on the inflammatory pathway and cholesterol-beta amyloid interactions has been taken. Cell number is dynamically modeled, changing in response to environmental cues for apoptosis or transmigration. Molecular interactions that have significant biological study and data to help define a pathway have been studied. Nodes were identified as significant if the biological data had identified a key role for them in the disease process, if nodes had a high degree and thus had a larger effect on downstream signaling, or both. The remainder of the paper will define the parameter space, discuss the details of the model, describe the network and corresponding equations, and give results of a basic simulation.

II. MODEL DEFINITION

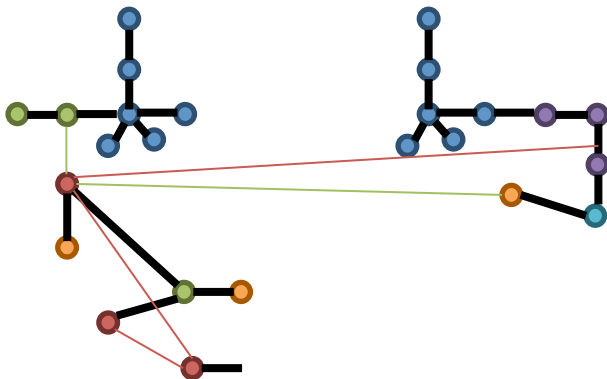


Figure 1: Network graph. Red nodes represent molecules involved in the inflammatory process; green nodes represent proteins; orange nodes represent an interaction with ApoE; blue nodes represent molecules involved in energy metabolism; light blue nodes represent protein-lipid complexes; and purple nodes represent lipid metabolism. Conservation of mass allows molecules to interact in more than one set of interactions. The graph drawn here is undirected, though directed edges were used in the modeling process.

A. Definition & Topology

The brain is composed of several key cell types, including neurons, microglia, astrocytes and brain endothelial cells. In this model, emphasis has been put on the metabolic interactions between these cell types; the physical processes of diffusion, mechanical or osmotic forces from plaques or excess local concentrations of chemicals, respectively, and the diffusion of neurotransmitter across synaptic clefts have been considered trivial. Each cell type has intrinsic pathways necessary for energy and protein production. All cell types are also able to produce lipids required for maintenance of their

cell membrane via the malonyl CoA pathway. In the adult brain, astrocytes synthesize cholesterol and transport it to neurons via the LRP-1 receptor.

A graph depicting the metabolic pathways was created to study the key intra- and intercellular interactions between different cell types within the brain. Nodes (denoted by capital letters *A*, *B*, etc) represent a particular chemical species, while edges represent the reactions or interactions (inhibition or activation, denoted by $\langle A, B \rangle$).

B. Nodal Analysis

The relative importance of each node was assessed by calculating the degree. The degree describes the number of edges coming into (degree in) or coming out of a node (degree out). A higher degree suggests that the node is key to the stability of the overall network since several paths converge on that node.

Table 1: Degree of nodes

Node	Degree In	Degree Out	Node	Degree In	Degree Out
APP	1	1	pyruvateA	1	1
AβN	3	3	ACoA_A	1	3
PyruvateN	2	1	HmgCoA_A	1	2
ACoAN	2	3	Mevalonate	2	2
Ach	2	1	CholA	2	2
CholN	1	1	ApoE	1	1
IL-1	4	5	ApoE*Chol	1	1
LRPN	2	1	AβECS	1	3
ATPN	3	0	Aβ_EC	1	2
ATPM	3	0	LRP_EC	2	0
TNFα	3	2	IL-6	1	2

III. MODEL EQUATIONS

The following paragraphs describe in further detail the equations and methods that were used to model the above network. Metabolic reactions were simplified to represent only the most relevant nodes of the pathway. Glucose levels are directly proportional to pyruvate levels in many cell types as the reaction has a relatively large $-\Delta G$ with several irreversible phosphorylation events driving the reaction forward. Pyruvate is transformed to acetyl coenzyme A via the action of pyruvate dehydrogenase (PDH), an enzyme that has been shown to be inhibited by beta amyloid [5, 22]:

$$ACoAN(n+1,1) = ACoAN(n,1) + pyrN(n,1) - 0.9ACoA(n,1) - 0.1ACoAN(n,1) \quad (1)$$

From acetyl coenzyme A (ACoA), the network branches to include paths to the citric acid cycle (TCA), the malonyl coenzyme A pathway and to acetylcholine generation in neurons, or cholesterol generation in astrocytes. TCA is the major source of cellular ATP, producing 12 ATPs per acetyl

CoA molecule. The transition from pyruvate to acetyl CoA produces an additional 3 ATP molecules per pyruvate. Complex IV of the electron transport chain has been shown to be inhibited by elevated beta amyloid levels [5], and has been incorporated here as an inhibitor of ATP generation (denoted by r_6):

$$\text{ATPN}(n+1,1) = 0.05r_6\text{ATPN}(n,1) + 12\text{ACoAN}(n,1) + 3\text{pyrN}(n,1) \quad (2)$$

Cholesterol generation occurs mainly by astrocytes in the adult brain since it is such an energy intensive process. Cholesterol production proceeds from HmgCoA through the mevalonate pathway through many precursors (not modeled here). Cholesterol is an important starting molecule for further reactions that generate other steroid molecules, including cortisol and the sex hormones. Elevated levels of cholesterol inhibit the action of HmgCoA reductase activity on the conversion of HmgCoA to mevalonate. Its transport to neurons is dependent on the levels of the ApoE and LRP proteins [14,17]. ApoE (apolipoprotein E) acts as a carrier molecule between astrocytes and neurons, while the LRP (low density lipoprotein-related protein) transports cholesterol into neurons [8]. Interestingly, LRP is also responsible for the majority of transport of beta amyloid out of the brain and into the plasma. This relationship has been modeled here by the following two equations, depending on whether ApoE or LRP is the dominant molecule:

$$\begin{aligned} \text{cholN}(n+1,1) &= 0.05\text{cholN}(n,1) + \text{LRPN}(n,1) \quad (3) \\ \text{cholN}(n+1,1) &= 0.05\text{cholN}(n,1) + \text{apoechol}(n,1) \quad (4) \end{aligned}$$

Within the brain, the innate immune response is the primary inflammatory response to irritants [22], and was modeled by describing the levels of IL-1, TNF α and IL-6 with respect to each other and to the level of beta amyloid [2,11,15,26-28]. TNF α is produced predominantly by activated microglia, and has a variety of effects, depending on the concentration that it is expressed at. At low concentrations ($<10^{-9}\text{M}$), TNF α leads to local inflammation, stimulation of integrin expression by brain endothelial cells that leads to increased transmigration of leukocytes across the blood-brain barrier (BBB), and stimulation of endothelial cells and microglia to produce chemokines and cytokines, such as IL-1 [21,25]. At increased concentrations, TNF α acts on the hypothalamus, increasing the synthesis of prostaglandins and leading to fever. Prolonged increases in TNF α levels can lead to cachexia due to appetite suppression and decreased synthesis of lipoprotein lipase (necessary to remove lipids from LDL). If levels increase systemically, hypoglycemia may occur, as well as intravascular thrombosis. This has been represented by the following equation, where g_1 and g_2 represent the production of TNF α by brain endothelial cells and microglia, respectively:

$$\text{TNFalpha}(n+1,1) = g_2\text{M}(n,1) + 0.5\text{TNFalpha}(n,1) \quad (5)$$

Interleukin-1 (IL-1) is produced by activated microglia, astrocytes and brain endothelial cells. Increased levels of IL-1 also lead to increased integrin expression by brain endothelial cells and subsequently increased transmigration of leukocytes into the brain with differentiation into microglia [10,11]. IL-6

is known to inhibit the secretion of both IL-1 and TNF α , and has been included as a repressor in this model (r_5):

$$\begin{aligned} \text{IL1}(n+1,1) &= \text{IL1}(n,1) + \frac{g_1}{r_5}\text{EC}(n,1) + \frac{g_2}{r_5}\text{M}(n,1) \quad (6) \\ \text{IL6}(n+1,1) &= g_3\text{M}(n,1) + \text{IL6}(n,1) \quad (7) \end{aligned}$$

IV. RESULTS

Model equations were simulated using Matlab and were run for 10,000 time steps (each step corresponds to a single day). Nodes were updated at each time step in response to the values of surrounding connected nodes and the above equations. Initial values were set to zero for molecules that are present in low number (including A β and all inflammatory molecules) in this set of simulation runs. Cell number changed in response to the concentration levels of inflammatory molecules and beta amyloid levels.

The basic model conditions were run here to demonstrate the ability of the model to give reasonably accurate results. The basic simulation showed stable results that converged to reasonable values within the simulation window. Cell number was constant since no perturbations to the inflammatory state or beta amyloid level were modeled here. Beta amyloid levels varied depending on the stochastic generation rate. ATP levels initially decreased before converging, while IL-1 levels varied between 0 and 20 units depending on the A β level.

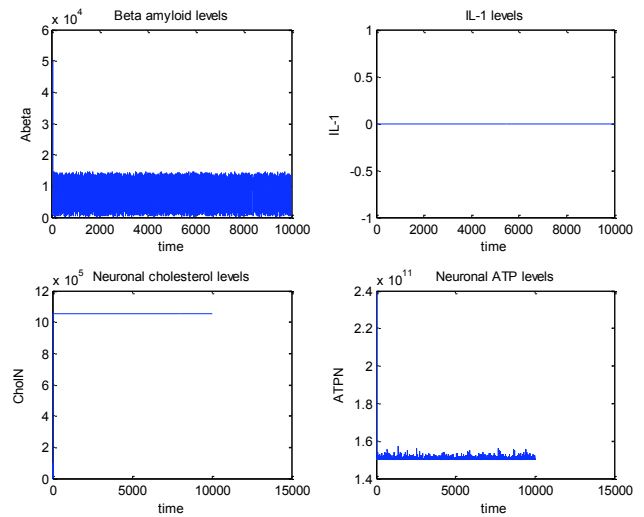


Figure 2: Simulation results of key molecules. Beta amyloid, IL-1, neuronal cholesterol and neuronal ATP levels were monitored. A β levels carried noise in transcription rates that were due to stochastic production rates.

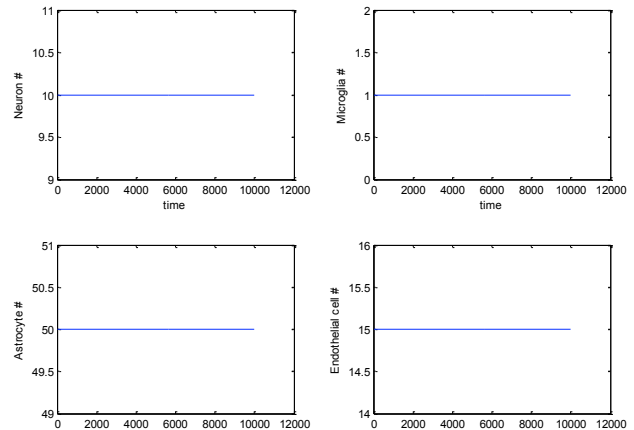


Figure 3: Simulations of cell number: The number of each key cell type was dynamically modeled and is able to be altered depending on the concentrations of IL-1, TNF α and A β .

Neuronal cholesterol levels were relatively constant.

The role of increased levels of inflammatory molecules due to either increased beta amyloid levels or independent increases in the levels of the pro-inflammatory molecules IL-1 and TNF α were also studied.

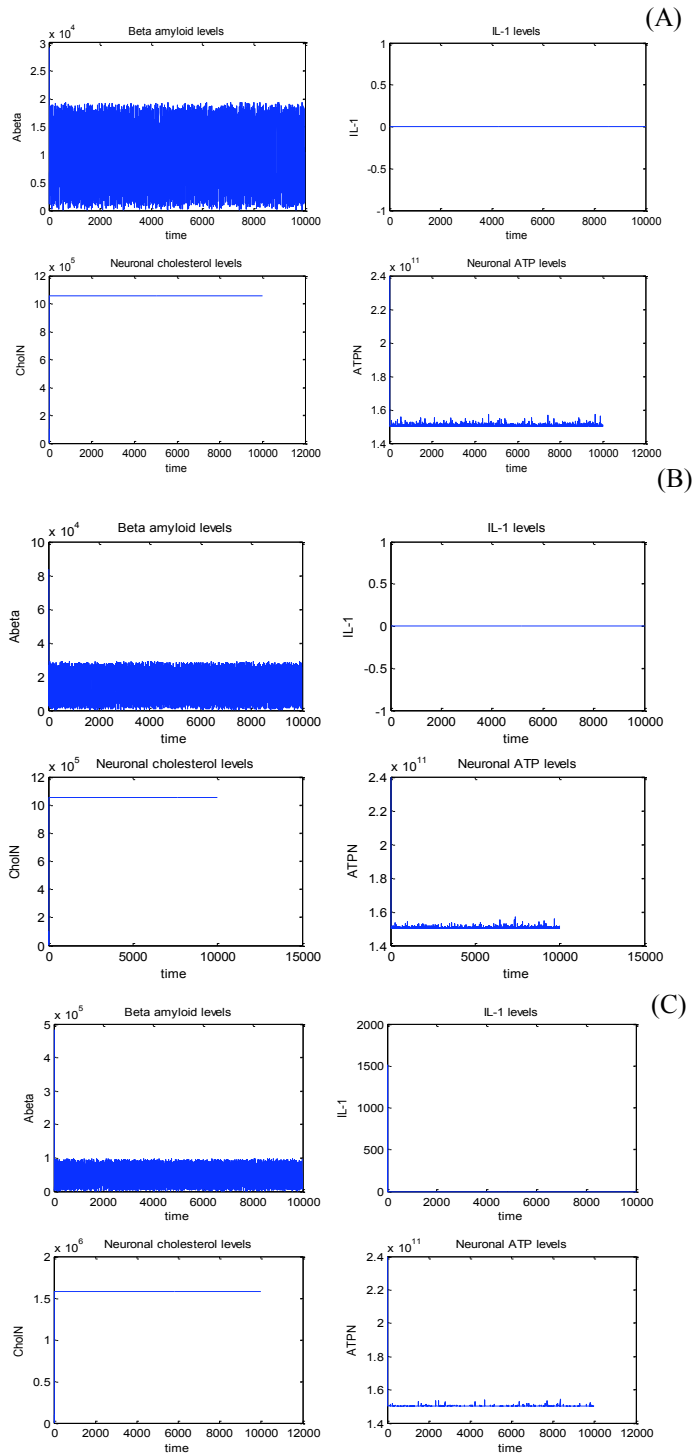


Figure 4: Simulations results of increased beta amyloid generation. (A) describes the effects of a 33% increase in A β generation, while (B) describe the effects of a doubling in the A β generation. Neither led to a significant change in any molecule other than beta amyloid. (C) describes a 100x increase in beta amyloid generation. In this case, neuronal cholesterol levels increase by a factor of about 10, while neuronal ATP levels lose much of the variability that they had in the control simulation.

Increasing the rate of generation of beta amyloid by 33% led to a mild increase in average and peak A β levels as expected. No significant differences between the control simulation levels and the modified simulation values were observed for IL-1, neuronal cholesterol and neuronal ATP levels. Cell number did not vary either (figure not shown). When beta amyloid generation rates were increased 100-fold, neuronal cholesterol increased approximately 10-fold. Neuronal ATP levels also lost some of the variability in levels that had been seen with previous simulations. As expected, microglia doubled in number (from one respective microglia to two microglia).

The effect of the pro-inflammatory molecules IL-1 and TNF α derived from sources external to the immediate system was also studied by introducing a short-term, single stimulus (10 time steps) and a long-term single stimulus (100 time steps). For the single, short-term stimulus, neuronal cholesterol peaked around the initial rise in IL-1 and TNF α and remained there for the remainder of the simulation. Interestingly, beta amyloid levels decreased to a lower average value after the short pulse of inflammation, while neuronal ATP levels increased slightly (~5%) though not significantly. Microglia number doubled and also remained increased for the remainder of the simulation.

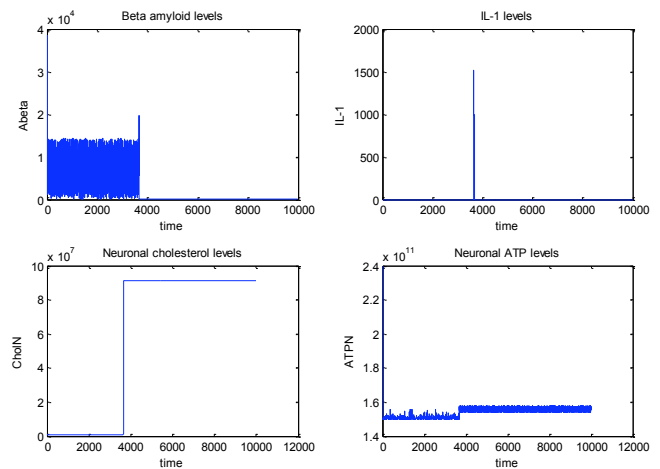


Figure 5: Simulations results of short term single stimulus of pro-inflammatory molecules IL-1 and TNF α from an external source.

For the long term increase in pro-inflammatory molecules, beta amyloid levels decreased significantly immediately following the stimulus before slowly increasing upwards. Neuronal cholesterol levels peaked temporarily around the initial rise in IL-1 levels, before returning to pre-stimulus levels. IL-1 levels increased significantly throughout the remainder of the simulation with a periodic trend as the number of microglia also increased. The number of neurons, astrocytes and endothelial cells did not vary during this simulation, though a longer simulation time may have shown a decrease in neuron number. Neuronal ATP levels were comparable to the control simulation.

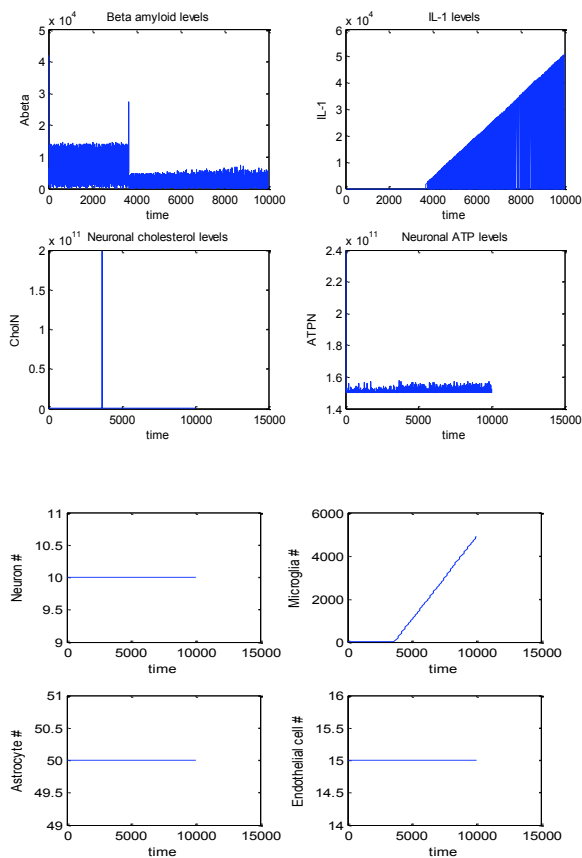


Figure 6: Simulations results of long term single stimulus of pro-inflammatory molecules IL-1 and TNF α from an external source.

V. DISCUSSION & CONCLUSION

The basic simulation results demonstrated a model that was stable for a range of input values. As expected, all values of inflammatory molecules were negligible over the duration of the simulation, though TNF α varied slightly between 0 and 20 units. All other molecules (proteins, lipids, molecules involved in glycolysis) converged within several time steps. In the basic simulation, the cell number does not change since there is no stimulatory input, though its ability to change was tested (not shown here).

Simulations studying the role of pro-inflammatory cytokines IL-1 and TNF α showed interesting results. Short-term inflammation can effectively be countered by the body and have little long-term effect on the level of neuroinflammation. However, these simulations suggest that both short term and long term inflammation may actually be beneficial to decreasing beta amyloid levels. However, long-term exposure to pro-inflammatory cytokines is not easily counteracted by the brain and can lead to a prolonged state of inflammation that gets progressively worse and recruits a significant number of microglia via transmigration. Though not studied here, if this trend were to continue, it could lead to decreased neuron number. Prolonged inflammation also triggered a slow increase in beta amyloid levels after the initial

decrease, which would have additional negative effects on neurons.

Mathematical modeling provides a useful method for better understanding the complex networks involved in the pathogenesis of AD. It allows the opportunity to manipulate the system with specificity that cannot be easily achieved using biological methods only. However, one of the drawbacks to modeling is the current lack of a sufficient amount of biological data derived from human trials to fit the model to, including the lack of consistent rate constants in the literature. This impasse is slowly being overcome as more databases with AD data are published and as better methods to study proteomic changes become available (for a good example of this see [44]). There is a significant need to perform a meta-analysis of currently available AD data to help determine which molecules and pathways play a key role in the pathogenesis process.

In this paper, a mathematical model was developed that describes the inter- and intra-cellular interactions between the four main cell types within the brain. This model specifically looks at the role of the inflammatory pathway in AD pathogenesis, and allows for monocytes to traverse the BBB and differentiate into microglia in response to increased levels of IL-1 and TNF α (a process known as transmigration). Cells can also enter apoptosis and leave the cell pool if ATP levels decrease below a certain threshold, allowing for dynamic modeling of cell number.

Future work will focus on further developing this model to include the role of cortisol and a more detailed inflammatory response. Stochastic rate constants will also be used in future models to better represent the probabilistic nature of transcription and translation. Simulations studying the role of these processes in further detail will also be completed to better understand the importance of the inflammatory process and altered cholesterol levels in AD pathogenesis.

Development of detailed mathematical models will open new avenues to personalized medicine. As this model develops into a more complex network, it will become increasingly useful to clinicians as a method to be able to predict the outcomes of individual patients as their disease progresses by taking into account genetic variations such as the ApoE4 allele or the presence of an underlying systemic inflammatory state like diabetes, thus allowing physicians the opportunity to tailor treatment and provide personalized medicine.

REFERENCES

- [1] Allan, S. M. and N. J. Rothwell. "Inflammation in central nervous system injury." *Philos Trans R Soc Lond B Biol Sci* 358(1438): 1669-77, 2003.
- [2] Arvin, B., L. F. Neville, et al. "The role of inflammation and cytokines in brain injury." *Neurosci Biobehav Rev* 20(3): 445-52, 1996.
- [3] Bjorkhem, I. and S. Meaney. "Brain cholesterol: long secret life behind a barrier." *Arterioscler Thromb Vasc Biol* 24(5): 806-15, 2004.
- [4] Butz, M., K. Lehmann, et al. "A theoretical network model to analyse neurogenesis and synaptogenesis in the dentate gyrus." *Neural Netw* 19(10): 1490-505, 2006.

- [5] Casley, CS, L Canevari et al. "Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities." *J Neurochem* 80(1):91-100, 2002.
- [6] Chauhan, N. B. "Membrane dynamics, cholesterol homeostasis, and Alzheimer's disease." *J Lipid Res* 44(11): 2019-29, 2003.
- [7] Craft, D. L., L. M. Wein, et al. "A mathematical model of the impact of novel treatments on the A beta burden in the Alzheimer's brain, CSF and plasma." *Bull Math Biol* 64(5): 1011-31, 2002.
- [8] Deane, R., A. Sagare, et al. "The role of the cell surface LRP and soluble LRP in blood-brain barrier A beta clearance in Alzheimer's disease." *Curr Pharm Des* 14(16): 1601-5, 2008.
- [9] Elias, P. K., M. F. Elias, et al. "Serum cholesterol and cognitive performance in the Framingham Heart Study." *Psychosom Med* 67(1): 24-30, 2005.
- [10] Giri, R, Y Shen et al. "Beta amyloid-induced migration of monocytes across human brain endothelial cells involves RAGE and PECAM-1." *Am J Physiol Cell Physiol* 279(6):C1772-C1781, 2000.
- [11] Grammas, P. "Neurovascular dysfunction, inflammation and endothelial activation: Implications for the pathogenesis of Alzheimer's disease." *J Neuroinflammation* 8(26) doi:10.1186/1742-2094-8-26, 2011.
- [12] Haass, C. and D. J. Selkoe "Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide." *Nat Rev Mol Cell Biol* 8(2): 101-12, 2007.
- [13] Halford, R. W. and D. W. Russell, "Reduction of cholesterol synthesis in the mouse brain does not affect amyloid formation in Alzheimer's disease, but does extend lifespan." *Proc Natl Acad Sci U S A* 106(9): 3502-6, 2009.
- [14] Hamanaka, H., Y. Katoh-Fukui, et al, "Altered cholesterol metabolism in human apolipoprotein E4 knock-in mice." *Hum Mol Genet* 9(3): 353-61, 2000.
- [15] Heneka, M. T. and M. K. O'Banion, "Inflammatory processes in Alzheimer's disease." *J Neuroimmunol* 184(1-2): 69-91, 2007.
- [16] Ledesma, M. D. and C. G. Dotti, "Amyloid excess in Alzheimer's disease: what is cholesterol to be blamed for?" *FEBS Lett* 580(23): 5525-32, 2006.
- [17] Liu, Q., C. V. Zerbinatti, et al, "Amyloid precursor protein regulates brain apolipoprotein E and cholesterol metabolism through lipoprotein receptor LRP1." *Neuron* 56(1): 66-78, 2007.
- [18] Luca, M., A. Chavez-Ross, et al, "Chemotactic signaling, microglia, and Alzheimer's disease senile plaques: is there a connection?" *Bull Math Biol* 65(4): 693-730, 2003.
- [19] Maccioni, R. B., J. P. Munoz, et al, "The molecular bases of Alzheimer's disease and other neurodegenerative disorders." *Arch Med Res* 32(5): 367-81, 2001.
- [20] Michikawa, M., Q. W. Fan, et al, "Apolipoprotein E exhibits isoform-specific promotion of lipid efflux from astrocytes and neurons in culture." *J Neurochem* 74(3): 1008-16, 2000.
- [21] Minghetti, L., "Role of inflammation in neurodegenerative diseases." *Curr Opin Neurol* 18(3): 315-21, 2005.
- [22] Pagani, L and A Eckert, "Amyloid-beta interaction with mitochondria." *Int J Alzheimer's disease*, 2011
- [23] Puglielli, L., R. E. Tanzi, et al, "Alzheimer's disease: the cholesterol connection." *Nat Neurosci* 6(4): 345-51, 2003.
- [24] Refolo, L. M., B. Malester, et al, "Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model." *Neurobiol Dis* 7(4): 321-31, 2000.
- [25] Rezaie, P. and D. Male, "Mesoglia & microglia--a historical review of the concept of mononuclear phagocytes within the central nervous system." *J Hist Neurosci* 11(4): 325-74, 2002.
- [26] Rossi, B, S Angiari et al, "Vascular inflammation in central nervous system disease: adhesion receptors controllin leukocyte-endothelial interactions." *J Leukoc Biol* 89:539-556, 2011.
- [27] Sabbagh, MN and DL Sparks, "Statins to treat Alzheimer's disease: an incomplete story." *Expert Rev. Neurother.* 12(1):27-30, 2012.
- [28] Sagare, AP, RD Bell et al, "Neurovascular dysfunction and faulty amyloid beta-peptide clearance in Alzheimer's disease." *Cold Spring Harbor Perspect Med.* doi: 10.1101/cshperspect.a011452, 2012.
- [29] Salminen, A., J. Ojala, et al, "Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence via pattern recognition receptors." *Prog Neurobiol* 87(3): 181-94, 2009.
- [30] Sastre, M., T. Klockgether, et al, "Contribution of inflammatory processes to Alzheimer's disease: molecular mechanisms." *Int J Dev Neurosci* 24(2-3): 167-76, 2006.
- [31] Selkoe, D. J., "Soluble oligomers of the amyloid beta-protein impair synaptic plasticity and behavior." *Behav Brain Res* 192(1): 106-13, 2008.
- [32] Streit, W. J., R. E. Mrazek, et al, "Microglia and neuroinflammation: a pathological perspective." *J Neuroinflammation* 1(1): 14, 2004.
- [33] Suo, Z., J. Tan, et al, "Alzheimer's beta-amyloid peptides induce inflammatory cascade in human vascular cells: the roles of cytokines and CD40." *Brain Res* 807(1-2): 110-7, 1998.
- [34] Takahashi, RH, CG Almedia et al, "Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain." *J Neurosci* 24(14):3592-3599, 2004.
- [35] Weksler, M. E., G. Gouras, et al, "The immune system, amyloid-beta peptide, and Alzheimer's disease." *Immunol Rev* 205: 244-56, 2005.
- [36] Wyss-Coray, T. and L. Mucke, "Inflammation in neurodegenerative disease--a double-edged sword." *Neuron* 35(3): 419-432, 2002.
- [37] Wyss-Coray, T, J Rogers, "Inflammation in Alzheimer disease- A brief review of the basic science and clinical literature." *Cold Spring Harbor Perspectives in Medicine* doi: 10.1101/cshperspect.a006346, 2012.
- [38] Yu, D, B Corbett et al, "Early cerebrovascular inflammation in a transgenic mouse model of Alzheimer's disease." *Neurobiology of Aging* doi: 10.1016/j.neurobiolaging.2012.02.02, 2012.
- [39] Ferreiro, E, I Baldeiras et al, "Mitochondrial and endoplasmic reticulum-associated oxidative stress in Alzheimer's disease: from pathogenesis to biomarkers." *Int. J Cell Bio* doi:10.1155/2012/735206, 2012
- [40] Eckert, A, K Schmitt et al, "Mitochondrial dysfunction- the beginning of the end in Alzheimer's disease? Separate and synergistic models of tau and amyloid beta toxicity." *Alzheimers Res Ther* 3(3):15, 2011.
- [41] Yao, J, RW Irwin et al, "Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease." *PNAS* 106(34): 14670-14675, 2009.
- [42] Parihar, MS & GJ Brewer, "Mitoenergetic failure in Alzheimer's disease." *Am J Physiol Cell Physiol* 292(1):C8-C23, 2007.
- [43] Robbins, SL, V Kumar & RS Cotran, "Pathologic Basis of Disease." Saunders/Elsevier, 8th edition, 2010.
- [44] Claydon, AJ, MD Thom et al, "Protein turnover: measurement of proteome dynamics by whole animal metabolic labelling with stable isotope labelled amino acids." *Proteomics* 12(8): 1194-1206, 2012.