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STUDYING THE ROLE OF ApoE IN ALZHEIMER'S DISEASE PATHOGENESIS USING A SYSTEMS BIOLOGY MODEL

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Alzheimer's disease (AD) is the most common form of dementia. Even with its well-known symptoms of memory loss and well-characterized pathology of beta amyloid (Aβ) plaques and neurofibrillary tangles, the disease pathogenesis and initiating factors are still not well understood. To tackle this problem, a systems biology model has been developed and used to study the varying effects of variations in the ApoE allele present, as well as the effects of short term and periodic inflammation at low to moderate levels. Simulations showed a late onset peak of Aβ in the ApoE4 case that lead to localized neuron loss which could be ameliorated in part by application of short-term pro-inflammatory mediators. The model that has been developed herein represents one of the first attempts to model AD from a systems approach to study physiologically relevant parameters that may prove useful to physicians in the future.

Keywords: Aβ (beta amyloid protein); AD (Alzheimer's disease); APP (amyloid precursor protein); ApoE (apolipoprotein E); ApoE ε2/3/4 (ApoE alleles); BACE (β-secretase); BBB (blood–brain barrier); IL (interleukin); LTP (long term potentiation); NMDA (N-methy-D-aspartate receptor); TNFα (tumor necrosis factor); TREM2 (triggering receptor expressed on myeloid cells).

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia in the elderly. It begins as the loss of short term memory and proceeds with the loss of long term memory as the disease progresses. Other symptoms of the disease include cognitive decline, depression, loss of voluntary muscle control and labile mood. At the histological level, AD pathology has been characterized by the presence of gross Aβ plaques and neurofibrillary tangles of hyperphosphorylated tau protein.[1] With the

increased size of the aged population in the Western world, the incidence of AD is expected to rise significantly in the next 30 years.

A variety of cellular changes have been observed in AD. The most prominent change is elevated Aβ levels, which forms the basis of the amyloid cascade hypothesis. The oligomeric form of Aβ has been shown to be neurotoxic and suppress long term potentiation (LTP) necessary to form memories.[2,3] Neuroinflammation has also been suggested to play a key role in the pathogenesis of the disease, however its role has not been determined as of yet. A connection between these two hypotheses does exist: Aβ has been shown to induce production of the pro-inflammatory cytokines interleukin-1 (IL-1) and TNFα, which stimulates further production of Aβ and creates a positive feedback loop until the Aβ is cleared.[4,5] Prolonged high levels of neuroinflammation have been shown to lead to synaptic dysfunction and neuronal loss, though low to moderate levels of pro-inflammatory cytokines can actually help ameliorate the levels of Aβ by increasing the clearance rate.[4] Inflammation is also balanced by the delayed production of interleukin-6 (IL-6) by microglia, which inhibits the generation of IL-1 and TNFα.

Dysregulation of lipid metabolism has also been suggested to play a role in AD pathogenesis.[6,7] Of particular importance is apolipoprotein E (ApoE), an apoprotein responsible for transporting cholesterol within the plasma, as well as from astrocytes to neurons within the brain.[6] ApoE has three alleles (ε2, ε3, ε4) that are commonly found in individuals, with the ε4 allele being associated with a significantly increased risk of late-onset AD. It is the only prevalent genetic risk factor for AD other than the recently identified TREM2 variants.[8]

Mitochondrial dysfunction with subsequent collapse of ATP levels and release of glutamate leading to production of reactive oxygen species (ROS) and cellular necrosis has been observed in AD.[9] Prolonged elevations of glutamate are also known to trigger release of cytochrome c from the mitochondrial membrane and trigger apoptosis.[9] The N-methyl D-aspartate (NMDA) receptor is known to play an important role in this process and has been implicated as a common convergent pathway for many other neurodegenerative disorders, including Parkinson's disease and Huntingdon's disease. Beta amyloid is also known to have an inhibitory effect on complex IV of the electron transport chain and pyruvate dehydrogenase (PDH), thus decreasing ATP levels.

Given the variety of complex mechanisms that may possibly be at play in the pathogenesis of AD can be an overwhelming task from the experimental biology standpoint. Mathematical modeling helps to define these interactions in more logical terms and allows for varying interactions in ways that are not easily possible using experimental techniques. By taking a systems biology approach, the model presented here builds on a model that we have previously developed, with important adaptations to study the role of the ApoE genotype on disease progress, and how genotype affects the response to inflammatory stimuli. Both cellular number and relative concentration of important chemical species has been modeled. Cellular number is a dynamic process that changes in response to environmental and chemical triggers.

This represents one of the first systems biology models available to study AD pathogenesis.

1.1. *Beta amyloid processing*

Beta amyloid (Aβ) is derived from the proteolytic processing of the amyloid precursor protein (APP) found on neuronal cell membranes and, to a lesser extent, on vesicular membranes. Cleavage of APP by β-secretase (BACE), subsequently followed by γ-secretase cleavage, results in the formation of Aβ. Cleavage by α-secretase leads to alternative, nonneurotoxic products.

Beta amyloid processing has been shown to be affected by the density of cholesterol in the plasma membrane, as well as the local inflammatory state. Experimental data has been mixed as to whether cholesterol levels in lipid rafts promote or inhibit Aβ cleavage.[6,10,11] In this model, we have assumed that excess serum cholesterol and low total brain cholesterol are positive risk factors for AD most related to the ApoE allele present.[12,13] The ε4 allele significantly decreases the ability of cholesterol and Aβ to interact with their respective receptors. In the brain, this leads to decreased neuronal cholesterol in addition to a decreased ability to clear Aβ from the brain via the blood–brain barrier (BBB). Additionally, experimental studies in an AD mouse model that we performed demonstrated that decreasing cholesterol through application of a statin lead to increased Aβ.[12]

1.2. *Cholesterol & ApoE*

Cholesterol is an extremely important molecule within the brain and serves important functions in synapse formation and maintenance, as a component of lipid rafts, in maintaining membrane stability, and in the synthesis of steroid-derived molecules such as cortisol. Dysregulation of cholesterol metabolism within the brain is associated with neuron loss and cognitive deficits.[6,14,15]

The majority of cholesterol is found within the plasma membrane and has a high affinity for associating with membranes containing sphingolipids and saturated phospholipids.[6] There are two known methods of cholesterol transport: vesicular and nonvesicular. Vesicular transport requires an intact cytoskeleton (microtubule tracts) and ATP to utilize motor proteins such as kinesin and dynein. Nonvesicular transport occurs via transport proteins such as ApoE. Within the brain, astrocytes are responsible for synthesizing the majority of cholesterol utilized by neurons, thus the nonvesicular pathway is believed to be the dominant method of cholesterol transport.

ApoE has three common variants depending on the single nucleotide polymorphism (SNP) present as defined above as ApoE ε2, ε3, ε4.[16] Studies have determined that the general allelic frequency varies with the population, with a frequency range of 6.4–10.1%, 61–78.3%, and 14.5–26.6% for ε2, ε3, ε4, respectively.[10] The majority of individuals are homozygous for ε3, with the next largest groups being ε3/ε4 and ε2/ε3.[10] Patients with the ε4 allele are at a significantly increased risk of getting AD, while patients with the ε3 allele have average risk and those with the ε2 allele are

slightly protected from AD. This may be due to the different binding capacities of the ApoE alleles; ApoE binds Aβ with an affinity of $\varepsilon 2 > \varepsilon 3 > \varepsilon 4$.[6] Tighter binding may allow Aβ to be more effectively cleared from the brain. These frequencies were taken into account during the simulation of this model.

1.3. *Neuroinflammation*

Inflammation has been a well-known process in AD and was even noted by Alois Alzheimer during his study of the disease that would carry his name. It is not well understood whether the inflammation is a result of the Aβ pathology, causes the Aβ pathology or works synergistically with Aβ to lead to the pathological state that is observed in AD. Experimental data has shown that the innate immune response is involved in the pathologic cascade via the following mechanism: Aβ stimulates the innate immune response via CD14 and Toll-like receptors (TLRs).[17] This in turn stimulates the production of pro-inflammatory cytokines such as IL-1 and TNFα.[5] Low levels of IL-1β have been shown to have a neuroprotective role via stimulation of Aβ clearance by activated microglia.[5]

Genetic association studies have demonstrated that individuals with AD have an increased inflammatory response to the presence of Aβ.[5] This response increases each time the patient is exposed to elevated levels of Aβ, which may precipitate an amplified and potentially neurotoxic inflammatory response.

2. Model Definition

In this model, a two level hierarchy of network interaction was developed. At the cellular level, the four main cell types (neurons, astrocytes, microglia, and brain endothelial cells) were allowed to interact with each other (Fig. 1). At the molecular level, each of these cell types had their own metabolic network that generated molecules related to their specific cellular function. For example, neurons are the main source for Aβ, which was modeled as neurons generating Aβ from APP cleavage. The products of the biochemical network of each cell type are then allowed to interact with the molecular products of other cellular networks in a process that allows modeling at the cellular and molecular levels.

The number of each type of cell was initialized (Table 1). The chemical species of each cell type was modeled by the average distribution and adjusted based on the number of cells of that type present. This method was chosen over modeling individual cells since it would be computationally difficult to model neural tissue at this level of detail given the sheer number of cells involved (on the order of millions of cells, even if looking at a relatively small volume of brain tissue). Additionally, this level of detail is unnecessary to model the general trends that would be seen by the interaction of these networks.

This model studied the interactions between several key parameters: Aβ generation, mitochondrial function, inflammation, and ApoE genotype. Rate constants

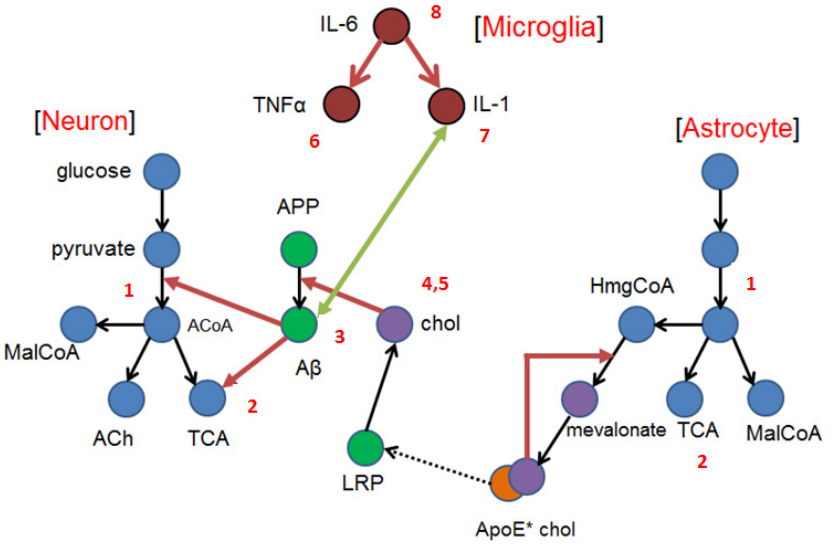

Fig. 1. Network graph. Red nodes represent molecules involved in the inflammatory process; green nodes represent proteins; orange nodes represent an interaction with ApoE; blue nodes represent molecules involved in energy metabolism; light blue nodes represent protein–lipid complexes; and purple nodes represent lipid metabolism. Conservation of mass allows molecules to interact in more than one set of interactions. The associated equations are indicated in the figure by the red numbers near the nodes. Glutamate is not shown in this figure.

and initialization values for key molecules are listed in Table 1. Model equations are discussed in further detail in the following paragraphs and the corresponding interaction in the network graph corresponds to the equation number.

2.1. *Model equations*

The following paragraphs describe in detail the equations and methods that were used to model the above network. The network described here was simplified to represent only the most relevant nodes of the overall pathway. Glucose levels are directly proportional to pyruvate levels given that the reaction has a relatively large $-\Delta G$ with several irreversible phosphorylation events driving the reaction forward. Pyruvate is transformed to acetyl coenzyme A (acetyl CoA) via the action of PDH, an enzyme that has been shown to be inhibited by beta amyloid.[18] The equation is written as amount produced per neuron and modified accordingly:

$$\mathrm{ACoA}_{n+1} = \mathrm{pyr}N_n\left(\frac{N_n}{N_1}\right). \tag{1}$$

The network branches from acetyl coenzyme A to include paths to the citric acid cycle (TCA), the malonyl coenzyme A pathway (fatty acid metabolism) and to acetylcholine generation in neurons (an important neurotransmitter). In astrocytes, it also branches to cholesterol generation via the mevalonate pathway. TCA is the

Table 1. Rate constants and variables used in the model. The initial values for key molecules, rate constants, and definitions for key variables are described. Relative rates and initial values were derived from those given in the literature.

Parameter	Initial value
Aβ	500
APP	100,000
Neuronal cholesterol	1×10^7
LRP	1×10^6
Neuronal ATP	2.5×10^{11}
IL-1	0
TNFα	0
Variables	**Represent**
ACoA_N	Acetyl coenzyme A
pyrN	Neuronal pyruvate
N	# of neurons at time n
ATP_N	Neuronal ATP
cholN	Neuronal cholesterol
Rate constant	**Value**
g_1	100
g_2	10
g_3	0.5
r_1	$\frac{1}{1+0.0001\mathrm{chol}N_n}$
r_5	$\mathrm{IL\text{-}6}_n$
r_6	$\frac{1}{1+\frac{\mathrm{AB}_n}{2}}$
a_1	1
ae_2	0.3
ae_3	0.32
ae_4	0.5

major source of cellular ATP, producing 12 ATPs per acetyl CoA molecule. The conversion of pyruvate to acetyl CoA produces an additional three ATP molecules per pyruvate. Complex IV of the electron transport chain is inhibited by elevated beta amyloid levels,[18] and is incorporated as an inhibitor of ATP generation (denoted by r_6):

$$\mathrm{ATP}_{N+1,n} = 0.05 r_6 \mathrm{ATP}_{N,n} + 12\mathrm{ACoA}_n + 3\mathrm{pyr}N_n, \tag{2}$$

where r_6 is the inhibition of ATP generation by Aβ. The amount of Aβ generated per time step was dependent on the number of neurons present and a stochastic cleavage rate of APP:

$$\mathrm{AB}_{n+1} = \mathrm{AB}_n + a_1 r_1 \mathrm{UAPP}_n - k\mathrm{AB}_n\left(\frac{N_n}{N_1}\right), \tag{3}$$

where r_1 describes the repression of Aβ production by cholesterol, a_1 is a function of the IL1 concentration (increased IL1 stimulates increased production of Aβ), U is a uniform distribution with mean 0.0015 and represents the stochastic generation of Aβ from differential interaction of the secretases with APP, and k is the degradation rate constant and is dependent on the expression level of LRP-1 on brain endothelial cells.

As discussed in the introduction, cholesterol generation occurs mainly by astrocytes in the adult brain. Cholesterol production proceeds from HmgCoA through the mevalonate pathway through a highly energy intensive process. Elevated levels of cholesterol inhibit the action of HmgCoA reductase, preventing the conversion of HmgCoA to mevalonate. Cholesterol transport to neurons is dependent on the levels of the ApoE and LRP proteins.[19] ApoE carries cholesterol from astrocytes to neurons, while the low density lipoprotein-related protein (LRP) transports cholesterol into neurons.[6,7,11] Interestingly, LRP is also responsible for the majority of transport of Aβ efflux from the brain.[20] This relationship has been modeled here by the following two equations, depending on whether ApoE or LRP is the dominant molecule:

$$\mathrm{chol}N_{n+1} = 0.05\,\mathrm{chol}N_n + \mathrm{LRP}N_n. \tag{4}$$

$$\mathrm{chol}N_{n+1} = 0.05\,\mathrm{chol}N_n + \mathrm{apoechol}_n. \tag{5}$$

The innate immune response is the primary inflammatory response within the brain,[5] and is modeled by describing the levels of IL-1, TNFα and IL-6 with respect to each other and to the level of beta amyloid. TNFα is produced predominantly by activated microglia and its effects are dependent on the concentration that it is expressed at. Low concentrations ($< 10^{-9}$M) of TNFα lead to local inflammation, stimulation of integrin expression by brain endothelial cells that leads to increased transmigration of leukocytes across the BBB, and stimulation of endothelial cells and microglia to produce chemokines and cytokines, such as IL-1.[5] At increased concentrations, TNFα acts on the hypothalamus, leading to the synthesis of prostaglandins and subsequently fever. Systemic increases in TNFα may lead to hypoglycemia and thus intracellular ATP levels. This has been represented by the following equation, where g_2 represents the production of TNFα by brain endothelial cells and microglia, respectively:

$$\mathrm{TNF}\alpha_{n+1} = g_2 M_n + 0.5\,\mathrm{TNF}\alpha_n e^{-n}. \tag{6}$$

Interleukin-1 (IL-1) is produced by activated microglia, astrocytes, and brain endothelial cells. Like TNFα, increased levels of IL-1 upregulate integrin expression by brain endothelial cells and subsequently increases transmigration of monocytes into the brain with differentiation into microglia.[4,5] IL-6 is known to inhibit the secretion of both IL-1 and TNFα, and has been included as a repressor in this model (r_5):

$$\mathrm{IL1}_{n+1} = \mathrm{IL}_n + \left(\frac{g_1}{r_5}\right)\mathrm{EC}_n + \left(\frac{g_2}{r_5}\right)M_n. \tag{7}$$

$$\mathrm{IL6}_{n+1} = g_3 M_n + \mathrm{IL6}_n, \tag{8}$$

where g_1 and g_2 describe the production of IL1 by endothelial cells and microglia, respectively; g_3 describes the generation of IL6 by microglia; and r_5 describes the inhibitory function that IL6 has on IL1 generation.

Glutamate neurotoxicity is another important component of the neuroinflammatory response present in AD. Elevated levels of glutamate are generated in response to cell injury, which in turn increases the intracellular levels of calcium via NMDA receptors. Elevated intracellular calcium levels stimulate oxidative phosphorylation, triggering the production of ROS that eventually lead to cell death.[9] Glutamate concentration levels were modeled by two cases:

$$\text{glut}_{n+1} = 10\,\text{glut}_n, \tag{9}$$

if ATP levels has decreased below a pre-defined threshold (5% of normal levels, which would happen within 1 min of loss of glucose/blood flow or ischemia from other sources). If ATP levels were normal or returned to normal after a stimulus, glutamate levels would exponentially decay:

$$\text{glut}_{n+1} = 0.02\ \text{glut}_n e^{-n}. \tag{10}$$

2.2. *Programming & simulation environment*

The model was coded and simulated using Matlab. The simulation was run for 10,000 time steps, where each time step represents a single day. Nodes were updated with each step with respect to changes in connected nodes as defined by the above equations. Feedback was included in the model to account for conservation of mass and internal feedback loops that are known to exist within these networks. Molecular number was initialized as described in Table 1. Cell number changed in response to the combined effects of ATP levels, Aβ levels and local concentration of pro-inflammatory cytokines above a specified level. Simulations were run to study the effects of the three ApoE variants on overall disease progression, and then expanded to study how short term and periodic inflammation affect this progression.

2.3. *Model robustness*

The robustness of the model was verified by varying the Aβ transfer rate constant (ae_2) from 0.1 to 0.35 and studying what happened to the Aβ and neuronal ATP levels. Simulations showed similar results up to $\text{ae}_2 = 0.35$, which was the expected transition point to the ApoE4 allelic variation (Fig. 2).

3. Results

3.1. *Reference simulation*

The initial simulation that was run studied the system response for a patient homozygous for ApoE3, the most common ApoE allelic variation in the general

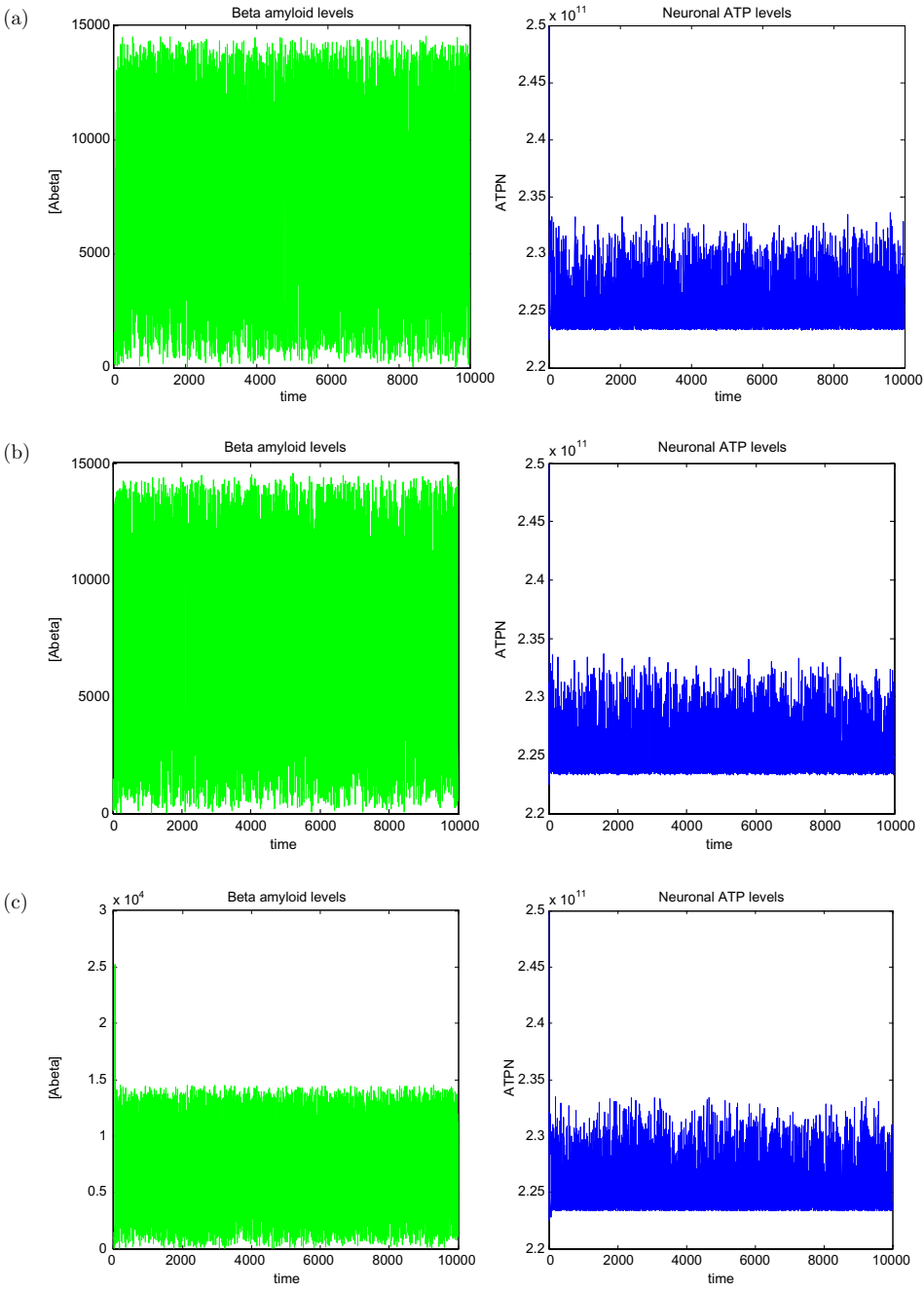

Fig. 2. Robustness simulations. (a) Reference simulation, (b) $ae_3 = 0.1$, (c) $ae_3 = 0.2$, (d) $ae_3 = 0.35$. Simulations show similar outcomes until (d), where the model transitions from the ApoE3 allele to the ApoE4 allele.

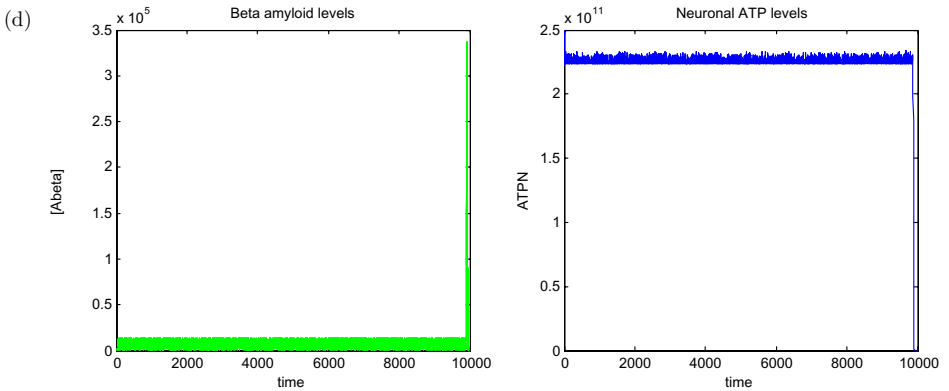

Fig. 2. (*Continued*)

population (Fig. 3). Cell number and molecular distribution was consistent with what was expected for this ApoE variant. Beta amyloid varied stochastically which gave rise to variations in the neuronal ATP levels that mirrored these changes (due to the inhibitory effects of Aβ on ATP generation).

3.2. *Effect of ApoE allele*

As stated previously, the ApoE allele contributes significantly to the rate of Aβ clearance across the BBB via the LRP-1 receptor. This effect on the progression of the disease was studied by varying the rate at which Aβ deposited in brain endothelial cells and contributed to the observed vascular Aβ load (Fig. 4). ApoE2/4 and ApoE3/4 alleles had quantitatively similar results.

3.3. *Effect of short-term, pulsed inflammation*

The effect of short-term inflammation was studied to determine if there was any alteration to disease pathogenesis when a pro-inflammatory state was simulated. A single, significantly elevated pulse of pro-inflammatory molecules (IL1, TNFα) was applied for a duration of 50 time steps (equivalent of 50 days) to model the effects of short term inflammation (Fig. 5).

3.4. *Effect of periodic inflammation*

Periodic elevations of pro-inflammatory molecules (IL1, TNFα) were modeled to study the interaction between ApoE allele and the sensitivity of the system to an inflammatory response [recall that biological experiments have shown that carriers of

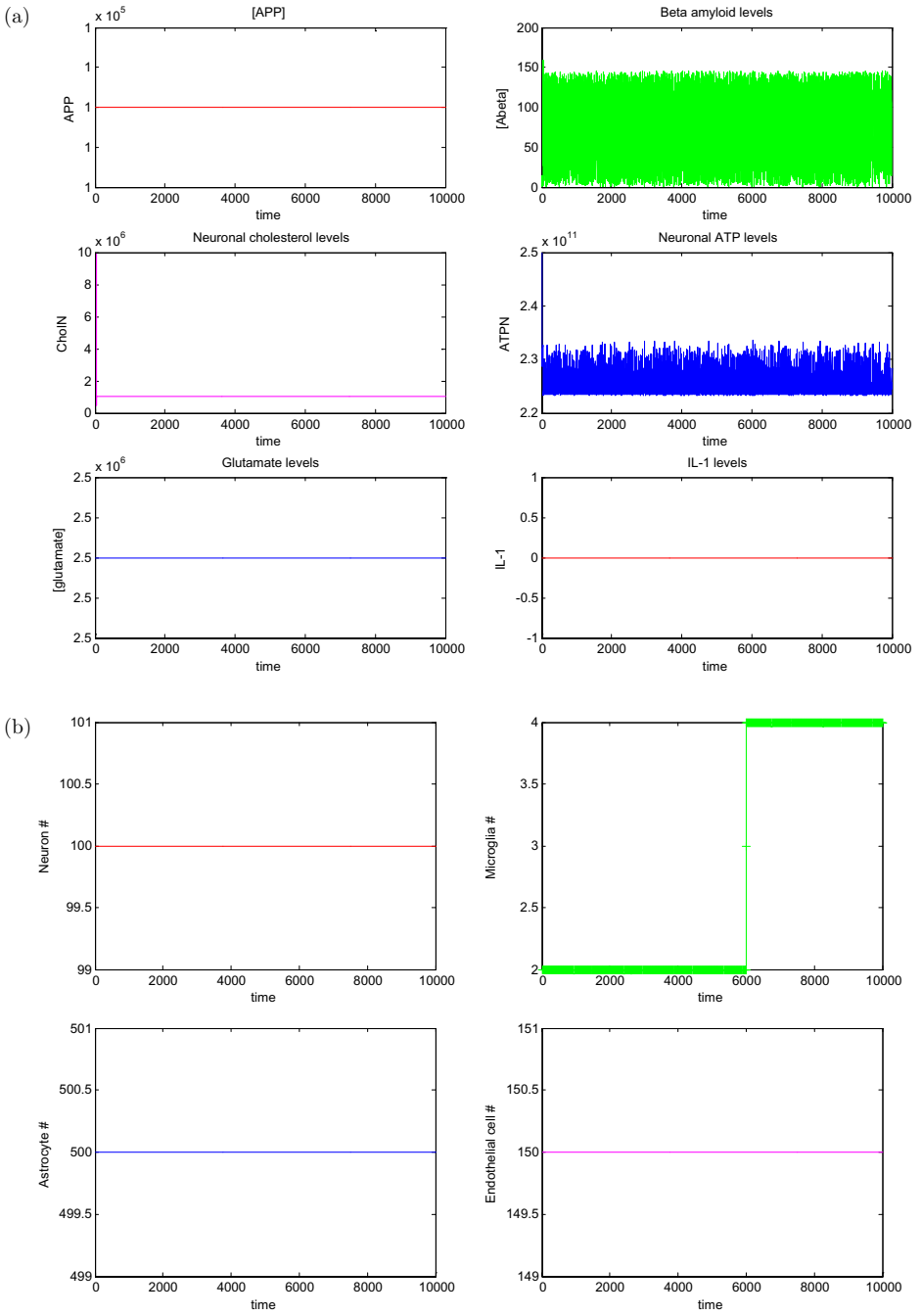

Fig. 3. Reference simulation. (a) (left column, top down) APP, neuronal cholesterol, and glutamate; (right column, top down) Beta amyloid, neuronal ATP, and IL-1; (b) cellular changes. Note that microglia increase slightly in response to the fact that the innate immune system increases its sensitivity as one ages.

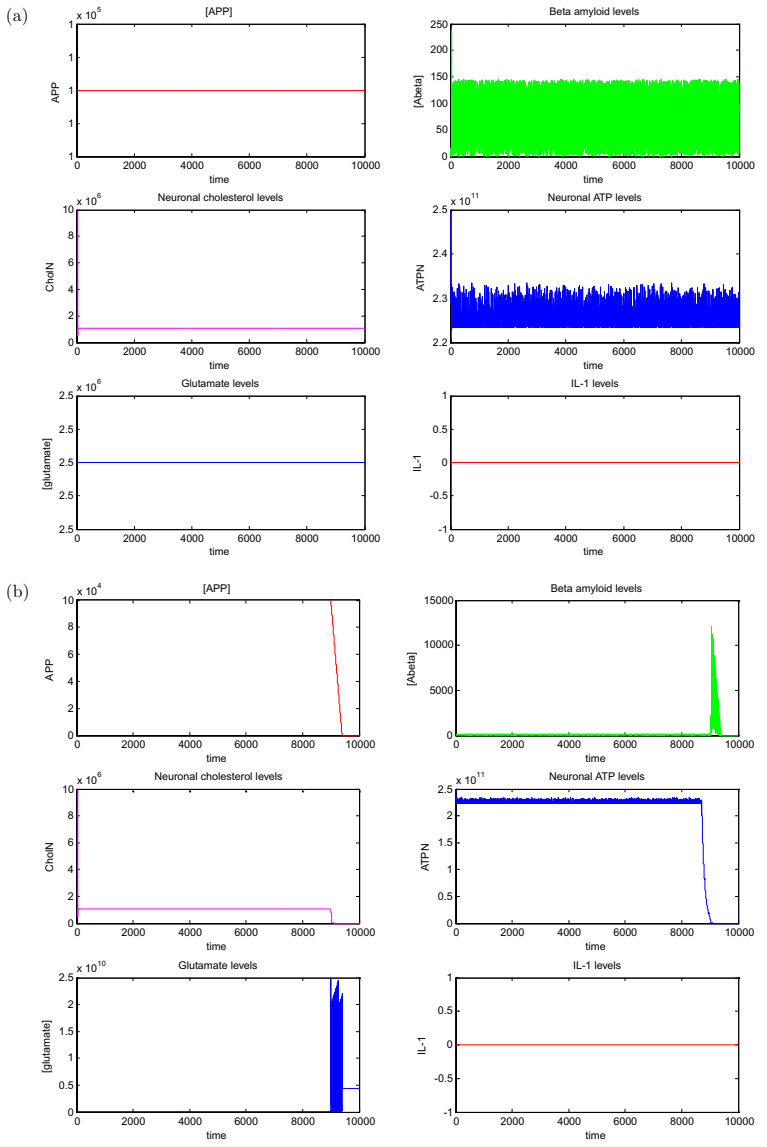

Fig. 4. Effect of ApoE alleles on disease pathogenesis. (a) ApoE3/3 homozygote is the reference simulation and demonstrates that there is no change in neuron number or beta amyloid (on average) over the course of the simulation. (b) ApoE2/4 heterozygote, demonstrates that an unsustainable peak in Aβ that occurs late in the disease process that leads to complete loss of neurons in the localized region that is modeled. (c) ApoE2/3 heterozygote demonstrates that the qualitative results are similar between the ApoE 3 homozygote and heterozygote and is sustainable over the course of time. (d) ApoE4/4 homozygote. This is the most severe case that is possible and is evident by the fact that neuronal cell death occurs earlier than the heterozygote case.

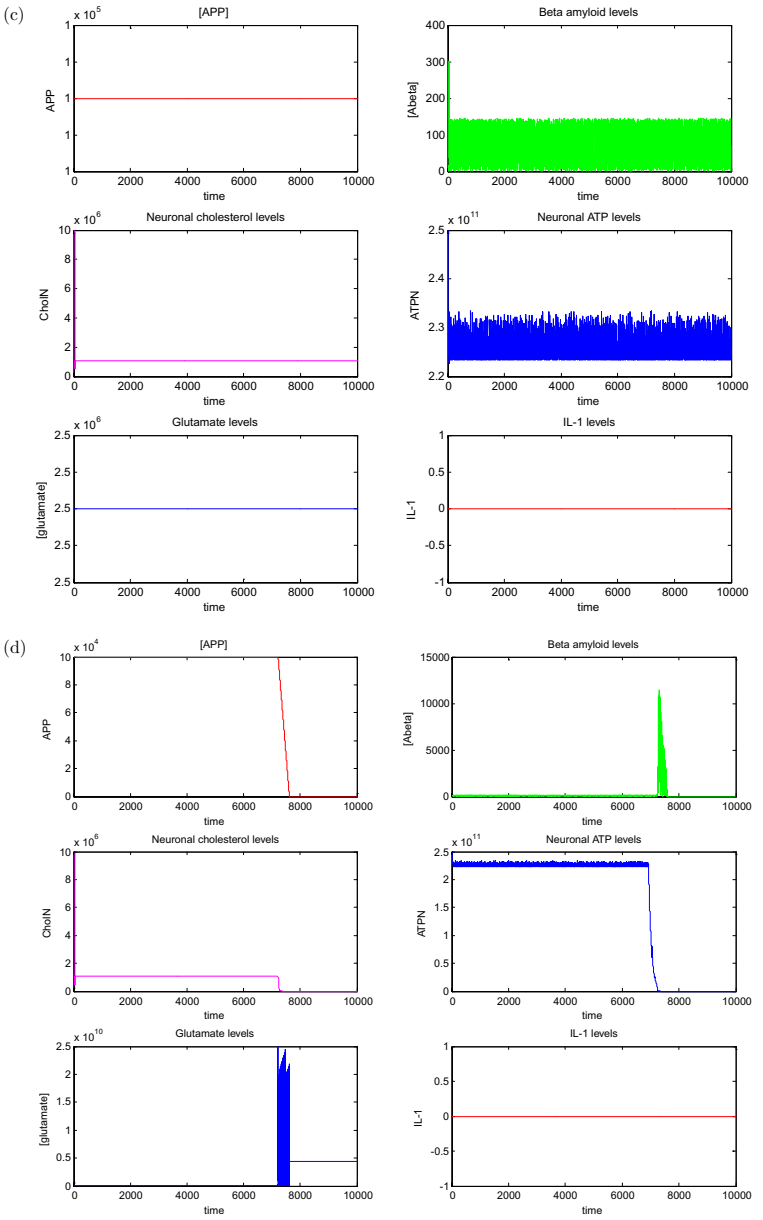

Fig. 4. (*Continued*)

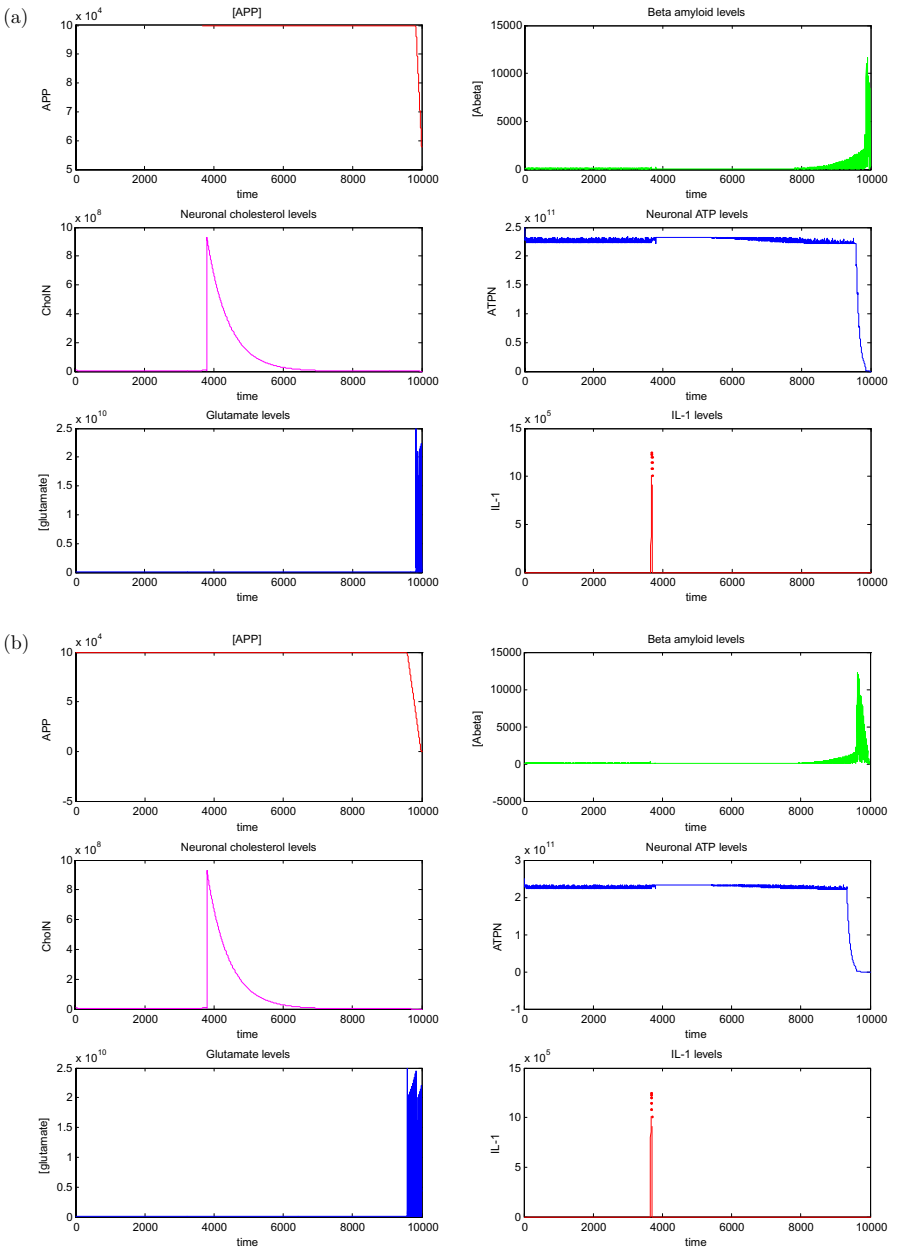

Fig. 5. Effect of a short-term inflammatory pulse on AD pathogenesis. (a) ApoE3/3 homozygote, (b) ApoE3/4 heterozygote, (c) ApoE4/4 homozygote.

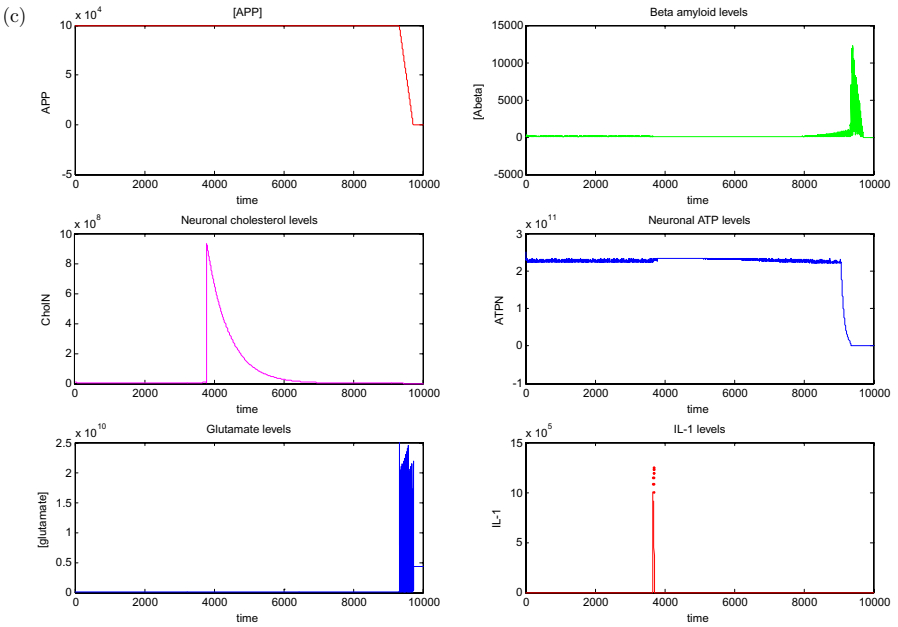

Fig. 5. (*Continued*)

the ApoE4 allele are more sensitive to the inflammatory response (Fig. 6)]. The single high strength inflammatory pulse modeled in Sec. 3.3 was followed by two very short inflammatory pulses. These pulses can biologically represent anumber of possible conditions affecting patients, such as meningitis, traumatic brain injury, major depressive episode, or other neuroinfection, all of which elevate the pro-inflammatory markers for a short duration.

3.5. *Effect of chronic, low level inflammation*

TNFα is produced by microglia in response to inflammatory stimuli such as Aβ and has been suggested to be a nonspecific marker of AD. As discussed before, low levels of TNFα stimulate the transmigration of leukocytes across the BBB, increasing the number of activated microglia present in the neural tissue to fight off infections or remove foreign material. Chronic low levels of TNFα were modeled to study whether there was a difference in response depending on the ApoE allele, as well as determine the strength of the inflammatory response in driving AD pathology (Fig. 7). The model allows for the transmigration of leukocytes and subsequent differentiation into microglia, temporarily increasing the number of microglia within the modeled brain region.

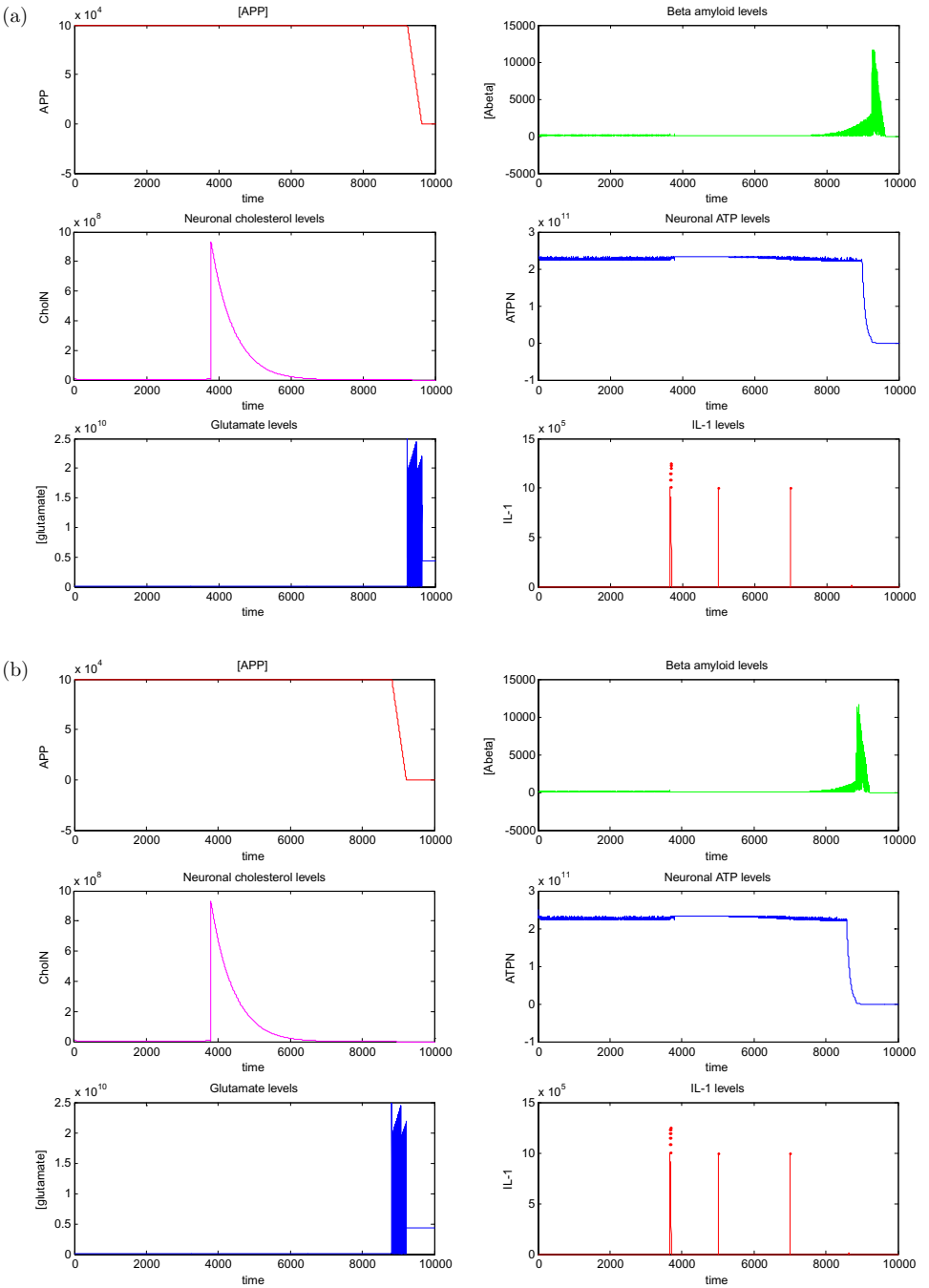

Fig. 6. Effect of a periodic inflammatory pulse on AD pathogenesis. (a) ApoE3/3 homozygote (b) ApoE4/4 homozygote.

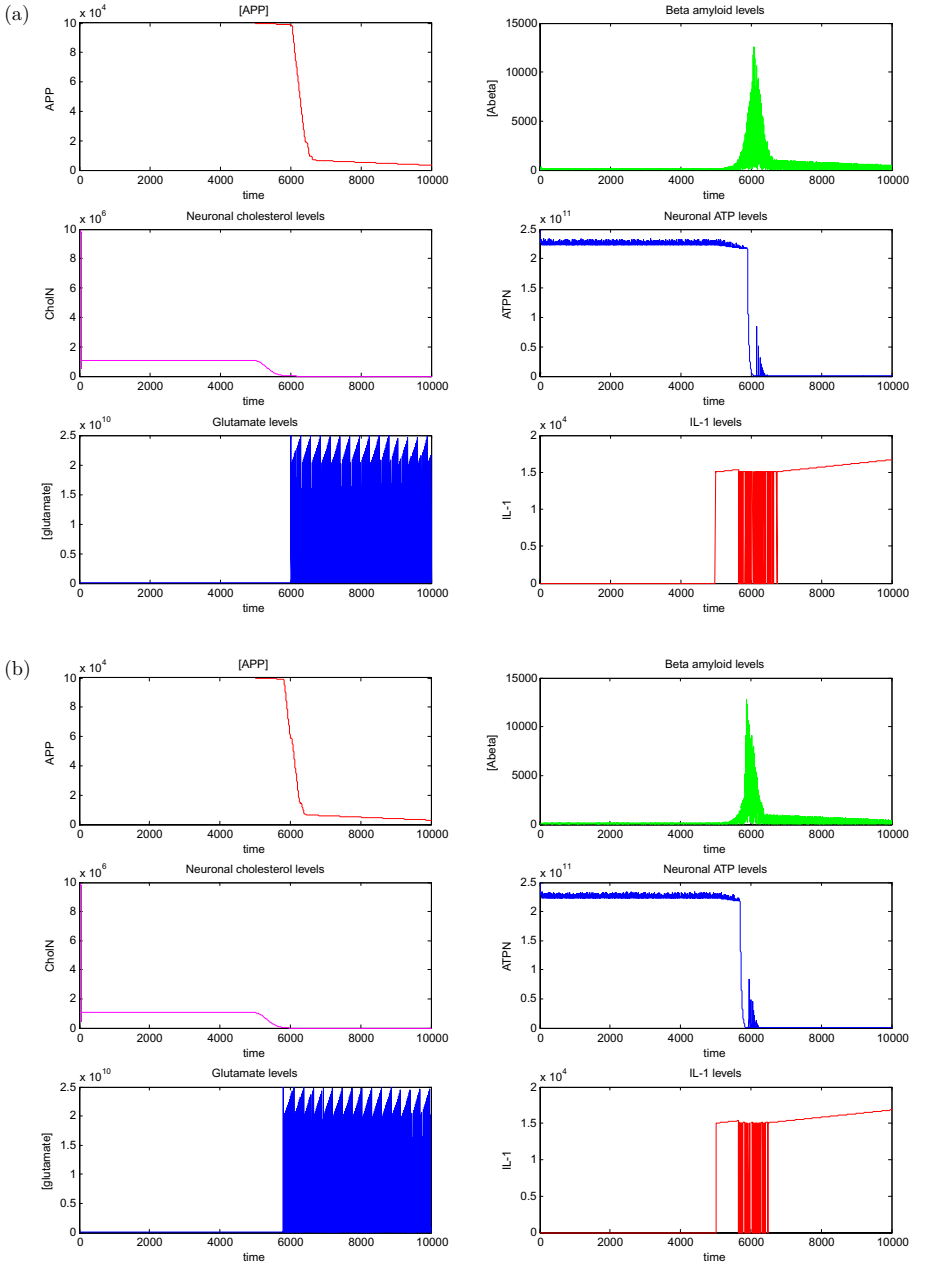

Fig. 7. Effect of a chronic, low level inflammation on AD pathogenesis. (a) ApoE3/3 homozygote (b) ApoE4/4 homozygote.

4. Discussion

In this paper, we have presented the results of several simulations run with the network model that we have developed. The system models the interactions between key molecules in inflammation, lipid metabolism, energy production, and Aβ production at both the molecular and cellular levels. The reference simulation demonstrated a stable network that is able to generate Aβ and ATP at stochastic rates (as defined by the model), while also modeling changes in the cellular microenvironment. Late onset increase in the innate immune system was modeled by increasing the transient number of microglia within the brain region being studied, as demonstrated by the slight increase in microglia number.

Simulations studying the effect of different ApoE alleles on the progression of disease demonstrated that having even one ApoE4 allele eventually lead to a significant local increase in Aβ that lead to the collapse of ATP levels and subsequent elevation of glutamate as is seen in experimental data. This was unsustainable, and led to the loss of all neurons in that local region over the course of an equivalent 1 to 1.5 years. This relatively abrupt loss of neurons was not ideal to model the neuronal loss actually seen in AD that often takes years to decades to develop such significant losses, and needs to be adapted in future models to better study this rate of neuronal loss. It is interesting to note that the collapse in ATP levels and loss of neurons was a late event in the simulations that was dependent on the allelic state of ApoE. ApoE4 heterozygotes fared better than ApoE4 homozygotes, but inevitably met the same fate. Qualitatively, this corresponds to the more severe risk for AD in those patients who are homozygous for ApoE4, with an increased AD risk in heterozygote patients.

Simulations on the effect of short-term inflammation showed that the level of neuronal cholesterol increased nearly immediately in response to the inflammatory response, regardless of the ApoE genotype, before returning to baseline levels. The elevated cholesterol levels suppress cleavage of APP into Aβ during this period, which subsequently eliminates the variability of neuronal ATP levels (this variability is a function of Aβ levels since Aβ inhibits PDH and thus ATP generation). Following this suppression of variability, ATP levels started to decrease slowly, which lead to a slow increase in Aβ. Eventually, this triggered glutamate toxicity that led to the collapse of ATP levels, followed by neuronal cell death. ApoE4 homozygotes appeared to benefit from this short inflammatory pulse by delaying the neuronal cell loss, while ApoE2 and 3 homozygotes appeared to be harmed in the long-term by the inflammatory pulse. This may seem a bit contradictory at first, but does make sense with known experimental data. The elevated cholesterol levels during the inflammatory pulse help to decrease the concentration of Aβ during this period, thus decreasing the amount of Aβ that will deposit in the brain parenchyma and at the BBB. This, in effect, delays the loss of neurons in those individuals with 1 or 2 ApoE4 alleles. In the case of individuals with ApoE 2 or 3, the delayed decreased levels of ATP due to the inflammatory pulse leads to a downward spiral of increased Aβ that at some point cannot be compensated for by degradation by microglia or

clearance by LRP-1. This in turn leads to collapse of ATP generation, glutamate toxicity, and neuronal loss.

Periodic inflammation as modeled by 3 peaks in IL-1 and TNFα showed a similar phenomenon as a single inflammatory peak, only shifting the time of neuronal cell loss early in the simulation period. The first peak, modeled as 50 time steps of inflammation (equivalent to 50 days of severe neuroinflammation) led to an increase in neuronal cholesterol levels similar to that seen in the short-term inflammatory simulation, however, subsequent, single day inflammatory peaks were unable to increase cholesterol levels and suppress Aβ generation. This suggests that inflammation may play a role in the AD process, but that the duration of the inflammation, as well as the strength of the inflammation, are important in determining whether the pro-inflammatory state will contribute, lessen or not even affect Aβ generation and AD progression.

Chronic, low level inflammation was modeled by elevating the level of TNFα for the duration of the simulation past a pre-assigned time point (in this case, for $n >= 5000$). At this level, TNFα increased the rate of leukocyte transmigration and differentiation into microglia, stimulating the initial increase in IL-1. This increase in IL-1 triggers IL-6 production, which has an anti-inflammatory effect and decreases the number of activated microglia and amount of IL-1 and TNFα. The initial prolonged increase in IL-1 also triggers an increase in the cleavage of Aβ, and it is this increase in Aβ that drives a return to the increased levels of IL-1, as well as inhibits PDH, decreasing the amount of Acetyl CoA that can be driven into the TCA and subsequently generate ATP. This collapse of mitochondrial function triggers the release of glutamate and, in combination with other pro-inflammatory markers, leads to neuronal cell death. In this simulation, the ApoE allele played little role in the final outcome; in all simulations, there was a collapse of ATP levels and loss of neurons. This agrees with biological data that has shown the brain is particularly sensitive to chronic inflammation and has little recourse to protect itself from a chronic assault.

5. Conclusion

In conclusion, we have presented a mathematical model to study the progression of networks involved in AD pathogenesis. The key cell types are able to interact at both a cellular and molecular level, with the network describing each cell type producing the functions and chemical species that are specific to that cell type. The roles of inflammation and the ApoE genotype were also studied, and demonstrated several important concepts, including the concept that a single inflammatory pulse in the brains of those carrying the ApoE4 allele may help protect against early neuronal cell death seen clinically by elevating cholesterol levels and effectively suppressing Aβ generation during the duration of the stimulus. This same inflammatory stimulus proves detrimental, however, if one instead carries the ApoE2 or 3 allele (without heterozygosity for ApoE4). Results of simulations with periodic inflammation

suggested that an inflammatory pulse needed to be sufficiently long enough to produce the cholesterol spike needed to suppress Aβ, demonstrating that the duration of inflammation may be more important than the acute degree of inflammation. This would be followed up on by studying the effect of chronic inflammation which again showed that the duration of inflammation was more significant than the ApoE genotype in causing elevated Aβ levels and subsequent neuronal cell loss.

There are still many adaptations and additional terms that need to be studied to develop a model fully capable of modeling the complex behaviors that occur in AD, however, this represents the first such attempt to create a multi-level network model of AD. In the future, such a model would offer a powerful complement to experimental research, and may offer physicians in the future the capability to better predict outcomes given specified lab values as inputs. Future work will continue to refine the model and include additional, relevant terms as more clinical data becomes available, as well as expand on the role of inflammation and ApoE allele on disease pathogenesis.

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