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Data-driven modeling of Alzheimer Disease pathogenesis

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ABSTRACT

Alzheimer Disease (AD) is the most prevalent form of dementia and the sixth leading cause of death in developed world. A substantial amount of data concerning the pathogenesis of this neurological disorder is available, but the complexity of the interactions they reveal makes it difficult to reason about them. This paper describes a computational model that represents known facts concerning AD pathophysiology and demonstrates the implications of those facts in the aggregate. The computational model is written in a mathematical language known as Maude. Because a Maude specification is an executable mathematical theory, it can be used not only to simulate but also to logically analyze the system it models. This model is based on the amyloid hypothesis, which posits that AD results from the build-up of the peptide beta-amyloid. The AD model represents beta-amyloid regulation, and shows through model analysis how that regulation can be disrupted through the interaction of pathological processes such as cerebrovascular insufficiency, inflammation, and oxidative stress. The model demonstrates many other effects that depend in complex ways on interactions between elements. It also shows how treatments directed at multiple targets could be more effective at reducing beta-amyloid than single-target therapies, and it makes several experimentally testable predictions. The work demonstrates that modeling AD as an executable mathematical theory using a specification language such as Maude is a viable adjunct to experiment, which allows insights and predictions to be derived that take more of the relevant biology into account than would be possible without the aid of the computational model.

1. Background

Back in the heyday of complex-systems studies, a great deal of attention was devoted to simple systems that exhibit complex behavior, because it was obvious that complex systems exhibit complex behavior. Nowadays more effort is devoted to understanding complex systems, which can be loosely defined as systems composed of many different parts that interact in many different ways. Biological systems are complex systems of this type, and the goal of systems-biological modeling is to develop methods to represent, simulate, and analyze them. Systems-biological modeling approaches are critical for understanding highly complex disease processes such as neurological disorders. The purpose of this paper is to describe a data-driven approach to modeling the pathophysiology of Alzheimer Disease (AD). As a complex disorder that is also the sixth leading cause of death in the developed world, AD is an apt subject for complex systems modeling.

A concept-driven (as opposed to a data-driven) model implements an assumed mechanism (e.g. amplifier, filter, servomechanism, multi-stable attractor, etc.) and demonstrates how the properties of that mechanism provide insight into a biological or other natural phenomenon. In contrast, a data-driven model represents a set of interrelated experimental findings and demonstrates the implications of those findings in the aggregate. In a data-driven model, the data are represented in terms of declarations that specify how interactions between variables should change the values of the variables. The declarations can be as quantitative as available data allow but, in the absence of precise measurements, the values of variables can change between, for example, multiple, arbitrarily assigned integer-valued levels. Assuming that the representation of the data is valid, a data-driven model can be used to analyze the properties of a naturally occurring system and to generate experimentally testable predictions, the results of which can be used to correct or extend the model.

Some of the first data-driven models of biological systems took the form of Boolean-logic networks (Thomas and D'Ari, 1990), and that approach is undergoing continued development (Klamt et al., 2006, 2007). Other approaches to data-driven modeling of biological systems include Petri nets, various process calculi, and hybrid models; those of the last type are composed of differential (or difference) equations, to simulate continuous changes in variables, and rules, to simulate switch-like changes in parameters (for reviews see Hlavacek et al., 2006; Fisher and Henzinger, 2007).

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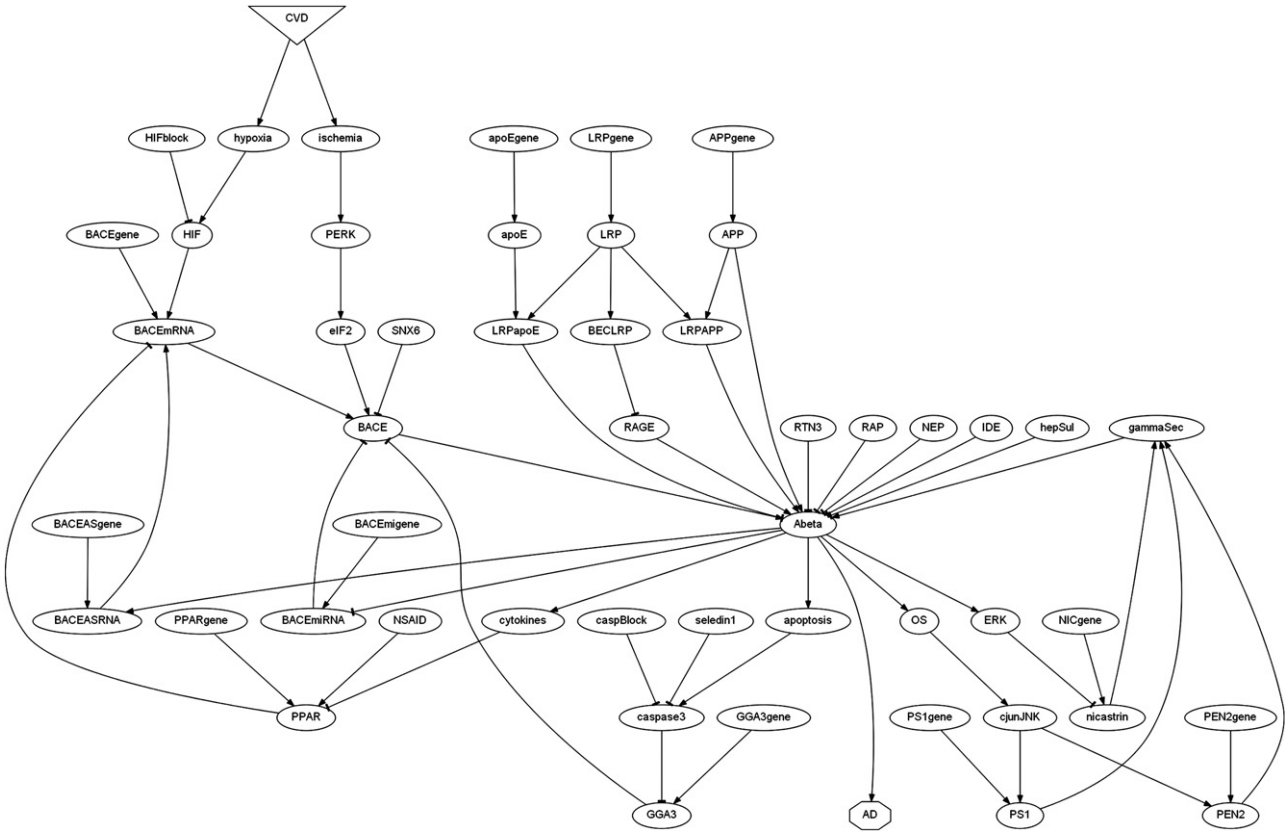

Fig. 1. Schematic of the data-driven model of Alzheimer Disease. The diagram depicts the influences that each element has on the other elements in the model. Arrowheads and tees represent positive and negative influences, respectively. The diagram was drawn using Graphviz software. The list of abbreviations is provided in Table 1.

The approach taken in this paper is most closely related to the Pathway Logic approach pioneered by Carolyn Talcott (Eker et al., 2002; Talcott, 2008). In Pathway Logic, molecular interactions are specified using a custom-designed graphical user interface to the Maude environment. The resulting Maude specifications are converted to Petri net models, which are then executed and analyzed using specialized Petri net tools.

The approach taken here is to represent interactions between molecules and conditions directly as declarations in Maude programs, and to use those programs as such in the Maude environment for simulation and analysis purposes. Maude is a mathematical modeling language in which systems can be modeled as sets of interrelated equations and rules. A Maude model constitutes an executable mathematical theory of the system it models (Clavel et al., 2007). Thus, a Maude model can be run as a computer program, to simulate the system it models, but also analyzed as a mathematical theory, to explore the properties of the system on a more general level. Because of its dual use potential, as a computer program and as a mathematical theory, a Maude model is known as a specification. Here Maude is used to specify a data-driven model of some of the interactions between molecules and conditions that are thought to underlie the complex etiology of Alzheimer Disease (AD). This Maude specification is used both for simulation purposes and as an object of analysis through state-space search and logical model checking.

Most of the data on AD biology is qualitative. To manage it, the molecules and conditions represented in the model are assigned arbitrary integer levels, and the equations and rules specify how changes in the levels of some model elements should change the levels of other elements. The model presented in this paper represents a small subset of the data on AD pathophysiology, but it is large enough that the nature of the interactions it models could not be discerned by inspection of the model diagram alone (Fig. 1). A longstanding goal of complex-systems studies has been to find general concepts that apply to whole classes of phenomena (Mainzer, 2007). In this spirit, we will show through simulation and analysis how the AD model reveals certain general properties that are likely to occur in real neurodegenerative disorders. However, the main focus here is on the details of AD etiology. Because all the variables are experimentally observable, simulation and analysis of the model automatically yields specific and testable hypotheses concerning the pathophysiology of AD. The results of the tests would have therapeutic value.

2. Methods

2.1. Biological background for the model

The model of AD pathophysiology is diagrammed in Fig. 1. The model is based on findings as reported in the primary literature. It is also, necessarily, based on a number of assumptions. The main assumption of the model is that AD results from build-up in the brain of the peptide beta-amyloid (or amyloid-beta, abbreviated Abeta). Although there are several other hypotheses concerning the cause of AD (see Section 4.3), the "amyloid hypothesis" is the leading hypothesis (Hardy and Selkoe, 2002). Efforts at AD therapeutics are directed both toward prevention and cure (Hardy and Selkoe, 2002). This model hopes to contribute to the former effort by focusing on the transition from the normal, healthy condition to the pathological state, rather than the state of advanced AD pathology in which brain levels of Abeta have been chronically elevated.

The Abeta peptide occurs in various lengths, but the 40 and 42 amino acid lengths are the most common (Selkoe, 2001). Although

Abeta-42 is more prone to aggregation, both Abeta-40 and Abeta-42 are toxic after they self-aggregate into oligomers and fibrils (Selkoe, 2001; Walsh and Selkoe, 2007; Di Carlo, 2010). For simplicity, no distinction is made between Abeta-40 and Abeta-42 in the model. The peptide Abeta is produced as a fragment of amyloid precursor protein (APP) due to cleavage of APP by two protease enzymes: beta-secretase (BACE) (Vassar et al., 1999, 2009; Cole and Vassar, 2007) and gamma-secretase (gammaSec) (Borchelt et al., 1996; De Strooper, 2003). The molecular biology and genetics of these protease enzymes have been the focus of intensive investigation over the past two decades (Selkoe, 2001; George-Hyslop and Petit, 2004; Chow et al., 2010).

The BACE enzyme exists as a monomer, but the gammaSec enzyme exists as a complex composed of the proteins known as presenilin-1 (PS1), presenilin enhancer-2 (PEN2), nicastrin, and anterior pharynx-defective phenotype-1 (APH1) (De Strooper, 2003). All four of these components can influence the activity, stability, and maturation of the gammaSec complex. Because little is known about the regulation of APH1 it is omitted from the model and assumed to be present constitutively.

Regulation of the other components of the gammaSec complex is represented in the model. Both PS1 and PEN2 are up-regulated by oxidative stress (OS). Specifically, OS, which is associated with the production of reactive oxygen species such as hydrogen peroxide, activates c-jun and the c-jun N-terminal kinase (cjunJNK) pathway, which in turn positively modulates gammaSec activity by up-regulating the expression of PS1 and PEN2 (Tamagno et al., 2008). In contrast, the extracellular signal regulated mitogen-activated protein kinase (ERK) pathway negatively modulates gammaSec activity by phosphorylating nicastrin (Kim et al., 2006). Build-up of Abeta leads to OS and activates the cjunJNK and ERK pathways (Kim et al., 2006; Bodles and Barger, 2005). Because the cjunJNK pathway up-regulates PS1 and PEN2 while the ERK pathway down-modulates nicastrin, these two pathways are thought to have a "dual opposite" effect on the expression of gammaSec (Tamagno et al., 2009). Both pathways are activated at high levels of Abeta but, because they essentially oppose one another, they do not change the level of gammaSec in response to increases in the level of Abeta (Tamagno et al., 2009). However, blocking the ERK pathway but not the cjunJNK pathway has interesting effects in the AD model (see Section 4.2).

Many mechanisms are known to regulate the expression and activity of BACE. These include both normative and pathological mechanisms. Some of these mechanisms allow BACE to drive its own expression through the production of Abeta. Two normative mechanisms, which are represented in the model, involve regulation at the RNA level. In one, Abeta increases the level of the beta-secretase antisense transcript (BACEASRNA), which stabilizes BACE messenger RNA (BACEmRNA) and increases BACE protein expression (Faghihi et al., 2008). In another, the microRNA-107 (BACEmiRNA) binds to a microRNA recognition element on BACEmRNA and reduces its translation to BACE (Wang et al., 2008). Because Abeta reduces expression of BACEmiRNA, BACE can reduce this negative influence on its own expression by producing Abeta (Wang et al., 2008). Thus, both of these normative loops constitute positive feedback up-regulation of BACE.

The model also represents two pathological positive feedback loops that up-regulate BACE. One involves build-up of Abeta leading to activation of inflammatory cytokines (Udan et al., 2008; Bulgarelli et al., 2009), which down-regulate the expression of peroxisome proliferator-activated receptor gamma (PPAR) (Sastre et al., 2006). Because PPAR down-regulates BACEmRNA expression, and because cytokines down-regulate PPAR, the activation of cytokines actually up-regulates the expression of BACEmRNA. In contrast, nonsteroidal anti-inflammatory drugs (NSAIDs) up-regulate PPAR expression and so down-regulate BACEmRNA expression (Sastre et al., 2006).

Another pathological positive feedback loop involves build-up of Abeta leading to apoptosis (Bader Lange et al., 2010). Apoptosis (the process of cell death) activates caspase-3 (caspase3), which then cleaves, and thereby inactivates, Golgi-localized gamma-ear-containing ADP-ribosylation-factor binding protein (GGA3; ADP is adenosine di-phosphate) (Tesco et al., 2007). GGA3 inactivates BACE by transporting it to the lysosome where it is degraded (Tesco et al., 2007). Activation of caspase3 by apoptosis inactivates GGA3 and thereby increases the level of the BACE enzyme. However, seladin-1 (seladin1) decreases caspase3 activity under apoptotic conditions (Sarajärvi et al., 2009). Like GGA3, sorting nexin 6 (SNX6) is another protein involved in trafficking BACE to the lysosome for degradation (Muhammad et al., 2008; Small, 2008).

An assumption that serves as an adjunct to the main assumption in the model is that incipient cerebrovascular disease (CVD) can act as a trigger for AD (Scheibel et al., 1989; de la Torre, 2009). The cerebrovascular insufficiency due to CVD causes both hypoxia (reduction in oxygen level) and ischemia (reduction in blood flow). These reductions are transduced into activated signaling molecules that can then affect the expression and activity of BACE. Two of these mechanisms are represented in the model. In one, the reduction in oxygen level activates hypoxia inducible factor-1-alpha (HIF), which increases the level of BACEmRNA (Zhang et al., 2007), probably by binding a BACE gene promoter and increasing BACEmRNA transcription (Guglielmotto et al., 2009). In another signaling mechanism, the energy deprivation due to ischemia activates pancreatic endoplasmic reticulum eIF-2-alpha kinase (PERK). Activated PERK phosphorylates eukaryotic initiation factor-2-alpha (eIF2), which then increases BACE translation by de-repressing a regulatory element on BACEmRNA (O' Connor et al., 2008).

The low-density lipoprotein receptor-related protein (LRP) is involved in several ways in the regulation of Abeta, and three of these are represented in the model. In the first, LRP bound to APP (LRPAPP) delivers APP to lipid rafts where it interacts with BACE and gammaSec, producing Abeta (Ulery et al., 2000; Yoon et al., 2007; Lakshmana et al., 2008). In the second, LRP bound to apolipoprotein E (apoE) clears Abeta. Specifically, apoE binds Abeta oligomers. Then the apoE/oligomer complex binds LRP at the cell surface, is internalized, and the Abeta is degraded by the lysosome (Strittmatter et al., 1993; Manelli et al., 2004). Since LRPAPP binds 1 molecule of APP, but LRPapoE binds Abeta oligomers (several molecules), the positive influence of LRPAPP on Abeta has absolute value 1 in the model, but the negative influence of LRPapoE on Abeta has absolute value 2 (see Section 2.2).

The third way in which LRP is involved in the regulation of Abeta concerns events that occur at the blood–brain barrier. LRP binds Abeta directly and transports it from the brain to the peripheral circulation (Shibata et al., 2000; Deane et al., 2004), while the receptor for advanced glycation end products (RAGE) transports Abeta in the opposite direction (Deane et al., 2003). Presumably these opposed effects are balanced in the normative state, and the model reflects this balance. The transport of Abeta occurs across brain endothelial cells (BECs), which are the cells that line cerebral blood vessels (Shibata et al., 2000; Deane et al., 2004, 2003). In the model, BECLRP represents the LRP that binds Abeta directly and transcytoses it across the BECs, while RAGE is represented simply as an element that increases the level of Abeta in the brain. The opposing actions of BECLRP and RAGE are represented by having BECLRP suppress RAGE. Thus, in the presence of BECLRP there is no net import of Abeta from the peripheral circulation to the brain, but RAGE is free to increase brain levels of Abeta in the absence of BECLRP.

Several other molecular mechanisms function to reduce the Abeta level, and some of these are represented in the model. Reticulon-3 (RTN3) colocalizes with BACE in the endoplasmic

reticulum and Golgi apparatus and inhibits Abeta production by binding with BACE (He et al., 2004; Kume et al., 2009). Similarly, heparan sulfate (hepSul) binds at or near the BACE active site and thereby also inhibits Abeta production (Scholefield et al., 2003). Receptor-associated protein (RAP) binds to Abeta and enhances its uptake, internalization by cells, and degradation (Kanekiyo and Bu, 2009). The enzyme neprilysin (NEP) is found in membranes, especially in lipid rafts, and it degrades Abeta intracellularly (Kanemitsu et al., 2003; Hiltunen et al., 2009). Insulin degrading enzyme (IDE) is primarily located in the cytosol, with smaller amounts present in membranes, but it also degrades Abeta intracellularly (Sudoh et al., 2002). These mechanisms reduce the Abeta level in the normative state.

Of course, all proteins must have genes that encode their amino acid sequences but, for clarity, the gene for a protein is included in the model only if a regulatory mechanism for that protein is also included in the model. The included genes are: APPgene, PS1gene, PEN2gene, NICgene, BACEgene, BACEASgene, BACEmigene, PPARgene, GGA3gene, LRPgene, and apoEgene. All of the interactions included in the model are represented, simulated, and analyzed computationally.

2.2. Computational representation, simulation, and analysis

In Maude, the specification of a model can be composed of one or more separate modules. The AD model is expressed in the Maude module `ALZHEIMER`. Each element of the model (condition or molecule) is represented in `ALZHEIMER` by an operator that assigns an integer-valued number to its level. In practice, all the levels are natural numbers (and many are just zero or one), but they are allowed to take integer values so that they can participate in integer operations (such as minus). The levels of the model elements (conditions and molecules) vary as a result of the interactions of their elements, as depicted by their connections in Fig. 1.

The connections drawn in Fig. 1 denote influence rather than binding. They represent either a positive (arrowhead) or negative (tee) influence. For example, the negative connection from PPAR to BACEmRNA means that PPAR reduces the level of BACEmRNA. The fact that it does this by altering the expression of the BACEgene is not represented in the diagram, but the effect of this interaction is represented in the module `ALZHEIMER`.

All connections have absolute value 1 unless information is available that indicates otherwise. For example, the negative connection from LRPapoE to Abeta has absolute value 2, because each molecule of LRPapoE removes several molecules of Abeta (Strittmatter et al., 1993; Manelli et al., 2004). In contrast, the positive connection from LRPAPP to Abeta has absolute value 1, because LRP binds only 1 molecule of APP. It then makes APP available to BACE and gammaSec in lipid rafts, thereby producing 1 molecule of Abeta (Yoon et al., 2007). The two-to-one ratio of the absolute effects of LRPapoE and LRPAPP is probably an underestimate, but the results concerning these species would only be enhanced using a higher ratio (see Section 3.5).

All of the influences are represented either as equations or as rules. In Maude, an equation must execute whenever it applies, and an equation applies whenever an existing term matches its left-hand side. A single equation can cause the Maude interpreter to loop if its right-hand side matches its left-hand side, and this problem is of particular concern in models in which the left-hand side contains variables, as they do in the AD model. To prevent looping in the AD model, all equations are conditional and apply only if the level of at least one model element will change as a result of execution of the equation.

As for an equation, a rule also applies whenever an existing term matches its left-hand side, but a rule may execute or not, and rules can execute in arbitrary orders. The rules in the AD model are also conditional, and this prevents them from executing when doing so would not change the level of any model element. The conditions in some conditional declarations (equations or rules) further specify that it should apply only if the level of Abeta exceeds a threshold. In most cases, conditional declarations with an Abeta threshold as part of the condition are declarations that affect elements that participate in feedback loops in the model.

The AD model contains two normative and four pathological feedback loops. The two normative loops allow Abeta to increase its own level. They include elements BACEASRNA and BACEmiRNA. BACEASRNA is an anti-sense RNA that stabilizes BACEmRNA and effectively increases its level, which in turn increases the level of the BACE enzyme. BACEmiRNA is a micro-interfering RNA that decreases translation of BACEmRNA to BACE. Expression of BACEASRNA and BACEmiRNA is enhanced and suppressed, respectively, by Abeta (see Section 2.1 for references). In the model, the level of BACEASRNA transitions from 1 to 2, and of BACEmiRNA from 2 to 1, whenever the level of Abeta is greater than 0.

The four pathological loops can be identified by their Abeta threshold elements. They are the ERK, OS, cytokines, and apoptosis loops. ERK is a protein kinase, OS is the condition of oxidative stress, cytokines is a generic name for molecules that mediate the inflammatory response, and apoptosis is the process of cell death. The thresholds of Abeta at which ERK or cytokines are activated, or at which OS, apoptosis, or AD occurs, are 5, 5, 5, 6, and 7, respectively. Note that ERK and cytokines are activated and OS occurs at the same threshold level of Abeta. These thresholds are ordered arbitrarily according to the rational that some conditions precede others in a disease process. Specifically, activation of ERK and cytokines, and production of the reactive oxygen molecules associated with OS, can be considered as signals for impending disruptions that should precede actual cell death (apoptosis), which should, in turn, precede the onset of AD symptoms.

In Maude, rules are used to express declarations that are concurrent in the sense that they can execute independently of one another (but not independently of equations). Specifically, Maude is designed so that all applicable equations execute first, then an applicable rule executes, then any equations execute that were made applicable by execution of that rule, then another applicable rule executes, and so on until no further equations or rules apply. Maude modules composed only of equations, or of equations and rules, can be used for simulation (Clavel et al., 2007). In the former case, a `reduce` command can be used to execute equations from some starting configuration (state) until no further equations apply. In the latter case, a `rewrite` command can be used to perform some pre-specified number of rule executions (during which equations execute as they apply). Simulations using these commands are useful for exploring the effects on some model element levels of changes in other levels.

Only Maude modules that include rules can be used for state-space search and temporal logic model checking, because those forms of analysis presuppose that events occur in a sequence. In Maude, events occur as rules are executed, and rules can execute or not, while equations implement required computations that must execute whenever they apply. Thus, only those state transitions that occur as a result of rule executions are those that occur in a sequence. To conduct a state-space search, Maude constructs the tree of state transitions resulting from all possible sequences of rule executions. Starting from an initial state, Maude executes every applicable rule, producing every possible one-step sequence, and from each of those resulting states Maude again executes every applicable rule, producing every possible two-step sequence from the initial state, and so on. Once Maude has constructed the tree of sequences, either to the terminus of each

sequence or to a pre-specified sequence length, it can search the tree for desired states. Temporal logic model checking can be thought of as a directed search through the tree of sequences (see Clavel et al., 2007 for details).

Depending on the simulation or analysis to be undertaken, different sets of declarations are expressed as equations or as rules in the AD model. The switch from equations to rules in module ALZHEIMER is used for two purposes. The first is to explore the interactions of the pathological loops in the model. To do this, the declarations that activate cytokines, and that cause apoptosis and AD, are expressed as rules (all other declarations are expressed as equations). Expressing these declarations as rules allows temporal logic analysis of pathological loop interactions. For example, using temporal logic analysis we can check whether cytokines are activated before AD occurs in the model.

The second switch from equations to rules is used to explore concurrency in the AD model. Concurrency could occur anywhere in the model, but it is especially interesting to explore concurrency at branch points. One branch point involves the up-regulation of PS1 and PEN2 by the cjunJNK pathway, and whether it occurs for both PS1 and PEN2, or for one but not the other, or for neither. PS1 and PEN2 are components of gammaSec, so their levels determine the level of gammaSec. To explore this branch point the declarations that specify the regulation by cjunJNK of PS1 and PEN2 are expressed as rules (all other declarations are expressed as equations). The other branch point concerns the fate of LRP, and whether it binds APP (LRPAPP), or binds with apoE (LRPapoE), or binds Abeta directly at the BECs (BECLRP), or all, some, or none of these. LRP and apoE are proteins involved in the trafficking of APP and Abeta, so the levels of LRPAPP, LRPapoE, and BECLRP influence the level of Abeta in the model. To explore this branch point the declarations that determine the levels of LRPAPP, LRPapoE, and BECLRP are expressed as rules (all other declarations are expressed as equations). The Maude search and logical model checking capabilities can be used to explore the consequences of executions of these rules in various combinations and orders.

3. Results

The Maude specification ALZHEIMER, along with a few Maude tools, was used to simulate and analyze those aspects of AD pathogenesis that the specification (model) represents. The approach essentially brings the data together and provides a view on what they mean in the aggregate. Although the data represented in the model constitute a small subset of the findings available in the literature, the diagram depicting the interactions that the model represents (Fig. 1) is too complex to be analyzed by inspection. Computational simulation and analysis leads to insights and experimentally testable predictions that could not be derived without the aid of the model. By way of organizing the results, those that concern the normal operation of the model are presented first, and further results are presented in the context of some common complex-systems concepts (Mainzer, 2007).

3.1. Normal behavior

All of the elements in the AD model can take any integer-valued level, but many elements simply take values of 1 or 0 indicating their presence or absence. In its normal state, all of the normative input elements take level 1 in the AD model while all of the pathological input elements take level 0. The input elements influence the non-input elements but are not themselves influenced by the non-input elements. Normative input elements include molecular species such as genes (APPgene, PS1gene, LRPgene, etc.) and proteins (IDE, NEP, RAP, etc.). The main pathological input element is incipient cerebrovascular disease (CVD), but pathological input elements also include artificial compounds (HIFblock, caspBlock, etc.).

The declarations in the AD model change the levels of the non-input elements according to the levels of the input elements and the other non-input elements. All version of the AD model have the same set of declarations. The different versions differ according to which declarations are rules and which are equations. The main difference between equations and rules is that equations must execute whenever they apply but applicable rules may or may not execute. In principle, therefore, the different versions of the model are not expected to have exactly the same behavior. In practice, the different versions of the AD model have largely the same behavior—the differences are small and do not affect the findings reported here. The normal behavior of all versions is the same. Most of the simulations and analyses are done using the version in which the declarations that activate cytokines, and that cause apoptosis and AD, are expressed as rules (all other declarations are expressed as equations). This is also the version used to begin the exploration of the AD model.

Model behavior can be explored by using it to simulate the production and regulation of Abeta. A simulation begins from an initial state in which the levels of the input elements are set to 1 (present) or 0 (absent), and the levels of the non-input elements are set to 0. The levels of the non-input elements are determined through simulation by the execution of equations and rules. A rule execution is known as a "rewrite". Because equations execute whenever they apply, and because equations can be made applicable by the execution of rules, the equations and rules together can be executed by issuing a rewrite command, which allows the rules to execute. The number of desired rule executions can be given as a parameter of the rewrite command. To establish a baseline, we first use the AD model to simulate the formation of Abeta in the normative state, in which incipient cerebrovascular disease (CVD) is absent (CVD(0)) but all genes and other normative input factors are present. To do this we issue a command to perform 20 rewrites from the initial state:

```
rewrite [20] in ALZHEIMER: PPARgene(1) PS1gene(1) PEN2gene(1) NICgene(1) GGA3gene(1) APPgene(1) LRPgene(1) apoEgene(1) BACEASgene(1) BACEmigene(1) BACEgene(1) seladin1(1) SNX6(1) hepSul(1) RTN3(1) RAP(1) IDE(1) NEP(1) CVD(0) NSAID(0) HIFblock(0) caspBlock(0) hypoxia(0) HIF(0) ischemia(0) PERK(0) eIF2(0) cytokines(0) PPAR(0) OS(0) cjunJNK(0) PS1(0) PEN2(0) ERK(0) nicastrin(0) gammaSec(0) apoptosis(0) caspase3(0) GGA3(0) APP(0) LRP(0) apoE(0) LRPapoE(0) LRPAPP(0) BECLRP (0) RAGE(0) BACEASRNA(0) BACEmiRNA(0) BACEmRNA(0) BACE(0) Abeta(0) AD(0).
```

Twenty rewrites are sufficient to reach a persistent, non-changing state. The result of twenty rewrites of the AD model from the normative input state is:

```
result State: PPARgene(1) PS1gene(1) PEN2gene(1) NICgene(1) GGA3gene(1) APPgene(1) LRPgene(1) apoEgene(1) BACEASgene(1) BACEmigene(1) BACEgene(1) seladin1(1) SNX6(1) hepSul(1) RTN3(1) RAP(1) IDE(1) NEP(1) CVD(0) NSAID(0) HIFblock(0) caspBlock(0) hypoxia(0) HIF(0) ischemia(0) PERK(0) eIF2(0) cytokines(0) PPAR(1) OS(0) cjunJNK(0) PS1(1) PEN2(1) ERK(0) nicastrin(2) gammaSec(1) apoptosis(0) caspase3(0) GGA3(2) APP(1) LRP(1) apoE(1) LRPapoE(1) LRPAPP(1) BECLRP(1) RAGE(0) BACEASRNA(2) BACEmiRNA(1) BACEmRNA(5) BACE(4) Abeta(4) AD(0).
```

Note that in the normative state conditions such as hypoxia, ischemia, and oxidative stress (OS) do not occur (hypoxia(0), ischemia(0), and OS(0)), and ERK and cytokines are not activated (ERK(0) and cytokines(0)). The gammaSec enzyme

reaches level 1 (gammaSec(1)), the BACE enzyme reaches level 4 (BACE(4)), and Abeta reaches level 4 (Abeta(4)), but AD does not occur (AD(0)). The lack of pathology in the normal state pertains because Abeta does not reach the thresholds of any of the pathological processes represented in the model. The other sections of Results will describe many cases where those thresholds are crossed.

Certain properties of the model can be checked in the normal state to ensure that it is working properly. An important check of model behavior concerns the input elements that represent the genes of the proteins that directly produce or that regulate the production of Abeta. Among the 23 input elements of the model there are 11 genes. Of those 11, one is the gene for APP (APPgene) and four are genes for the proteolytic enzymes that cleave APP to form Abeta. Clearly, if the model is working properly, then the absence of any of these five genes should preclude Abeta production in the model. The other six genes are for other proteins involved in the regulation of Abeta production. The absence of any of those genes may or may not preclude Abeta production in the model. Logical model checking was used to check which of these genes are necessary for the production of Abeta in the model.

Logical model checking begins by writing equations for the satisfaction of properties of interest in model analysis. A property of interest in checking the necessity of certain genes for the production of Abeta is aBeq0, which is true if the level of Abeta equals 0. An equation that specifies the condition under which the AD model satisfies this property is: eq AM(S Abeta(0)) $\models$ aBeq0 = true, where AM is an operator that wraps (contains) the state of the AD model and where all elements not of immediate interest can be subsumed under variable S. The symbol $\models$ stands for logical satisfaction. This satisfaction equation, along with many others, is included in the separate Maude module MC-ALZHEIMER. Module MC-ALZHEIMER can be used to model-check ALZHEIMER, which is done through execution of the satisfaction equations in MC-ALZHEIMER along with the logical model checking tools provided in the Maude environment (Clavel et al., 2007).

Execution of equations is known as "reduction" in the Maude environment (Clavel et al., 2007). The following reduce command checks that the Abeta level should always be 0 if the APP gene is absent: reduce in MC-ALZHEIMER: modelCheck(AM(APPgene(0)...), [] aBeq0), where [] stands for the temporal logic operator "always". Note that the "..." is not part of the actual command but simply signifies that most of the operators specifying initial element levels are not shown. For clarity, only the APPgene operator is shown in the reduce command, and it sets its element to level 0. The other operators set the level of their element either to the normative level, for input elements, or to 0 initially for the non-input elements. The result is Bool: true. Thus, the APP gene is necessary for the production of Abeta in the model, as it should be. Similar checks show that the genes for BACE, PS1, PEN2, and nicastrin are also necessary for Abeta production in the model.

These results prove that Abeta can only be produced in the model if the gene for its precursor, APP, and those for the proteins that cleave APP to form Abeta, are present. The results also confirm that the basic aspects of Abeta production are working as they should in the model. Although PPAR, GGA3, LRP, apoE, BACEASRNA, and BACEmiRNA all take part in Abeta regulation, none of their genes are necessary for Abeta production in the model because their absence does not preclude it. They nevertheless play crucial roles, as further results reveal.

3.2. Multifactoriality

Many biological systems are multifactorial in that their behavior depends in nonlinear ways on many interacting factors. Multifactoriality can be demonstrated in the AD model through a study of some of the factors that reduce Abeta levels in the normal brain. In the model, factors that reduce Abeta include IDE and NEP, which are proteases that degrade Abeta. Heparan sulfate (hepSul) is a proteoglycan and RTN3 is a protein, and both inhibit the binding of BACE and APP, thereby impeding Abeta formation. RAP binds Abeta and transports it to the lysosome for degradation (see Section 2.1 for references). Because quantitative information on their influences (such as accurate measurements of their effects on Abeta in the same whole animal or cellular preparation) is not available, the absolute values of the negative influences of IDE, NEP, hepSul, RTN3, and RAP are all set to 1. In the absence of quantitative information, assigning the same absolute value to all of these influences facilitates comparison of the effects of removing them.

To explore the effects of removal of IDE, its level is set to 0 (IDE(0)) but all other elements, including NEP, are maintained at their normative levels. As expected, this causes the level of Abeta to rise by 1 point, from the normative level 4 to level 5 (Abeta(5)). The same result is observed if NEP is removed but IDE is maintained at its normative level (NEP(0) but IDE(1)), that is, Abeta rises by 1 point to level 5 (Abeta(5)). In fact, the same result is observed if any one of IDE, NEP, hepSul, RTN3, or RAP is set to 0 with all the others set to 1.

Because each of IDE and NEP have a negative influence of strength 1 on the level of Abeta, removal of both IDE and NEP would be expected to increase Abeta by 2 points, from the normative level 4 to level 6. This is still below the Abeta threshold of 7 (strictly greater than 7) that is needed to produce AD in the model. The expected result does not occur. Instead, when the simulation starts with both IDE and NEP absent (NEP(0) and IDE(0)), Abeta increases by 4 points, from the normative level 4 to level 8, which crosses the AD threshold and produces AD (Abeta(8) and AD(1)). This nonlinear jump demonstrates multifactoriality in the AD model.

Multifactoriality in the model can be understood in terms of the thresholds that regulate the operation of pathways, and especially of loops, in the model. Removal of IDE or NEP alone increases the level of Abeta from the normative level 4 to level 5, but Abeta level 5 does not cross any of the pathological thresholds as set in the model (the lowest pathological thresholds are strictly greater than 5; see Section 2.2). By themselves, removal of both IDE and NEP would increase Abeta to level 6, but level 6 exceeds some of the pathological thresholds in the model. Crossing some thresholds could increase the Abeta level, which could then exceed other thresholds and further increase the Abeta level. That this occurs in the AD model can be analyzed using temporal logic model checking, because the declarations that represent these pathological threshold crossings are expressed as rules in this version.

A property of interest in exploring multifactoriality is hasAD, which is true if the model develops AD (as when AD reaches level 1). An equation that specifies the condition under which the model satisfies this property is: eq AM(S AD(1)) $\models$ hasAD = true. Other properties of interest include cytACT and hasAPO, which are true if cytokines are activated or if apoptosis occurs, respectively. These equations are also included in the separate module MC-ALZHEIMER.

We can use the Maude model checker to check that, in the model, AD does not occur until cytokines are activated. The command for this check takes the form: reduce in MC-ALZHEIMER: modelCheck(AM(IDE(0) NEP(0)...), ~(hasAD) U cytACT). Note that ~ and U stand for the temporal logic operators "not" and "until", respectively. Also, for clarity, only the IDE and NEP operators are shown in the reduce command, and both set their element to level 0. The other operators set the level of their element either to its normative value, for input

elements, or to 0 initially for the non-input elements. The result of this reduction is true. This proves that AD does not occur until cytokines are activated in the model.

As shown in the model diagram (Fig. 1), cytokines inhibit PPAR, which is a nuclear receptor protein that, among other things, decreases transcription of the BACE gene and so reduces the level of BACEmRNA. Because cytokines inhibit PPAR they inhibit an inhibitor of the BACE gene and so increase the level of BACEmRNA. This increases the level of the BACE enzyme, which increases the level of Abeta. Thus, removal of both IDE and NEP increases Abeta enough to produce AD in the model, but that does not occur until cytokines are activated.

We can use model checking to analyze the other pathological loops in the model. The thresholds for activating ERK and OS are the same as that for activating cytokines, and similar analyses show that AD does not occur in the model until ERK and OS are activated. Is this enough to cause AD in the model? No, as revealed by the following checks. The command `reduce in MC-ALZHEIMER: modelCheck (AM(IDE(0) NEP(0)...), ~(hasAD) U hasAPO)` checks whether apoptosis must occur before AD occurs in the model, and the result is true. Thus, the apoptosis loop as well as the other loops must be brought into play in order for AD to be produced in the model. The command `reduce in MC-ALZHEIMER: modelCheck (AM(IDE(0) NEP(0)...), ~(hasAPO) U cytoACT)` checks whether cytokines must be activated before apoptosis occurs in the model, and the result is again true. This proves the following chain of events in the model: removal of both IDE and NEP raises Abeta enough to activate cytokines, which further raises Abeta enough to cause apoptosis, which further raises Abeta enough to cause AD. Thus, multifactoriality in the model is a compound threshold effect.

3.3. Sensitive dependence

Many complex systems exhibit sensitive dependence, in which small changes in initial or ongoing conditions can cause large changes in other system variables. A proverbial example is the beat of a butterfly wing that precipitates a change in the weather (Mainzer, 2007). Many studies suggest that incipient cerebrovascular disease (CVD) too minor to cause overt pathology on its own can nevertheless precipitate AD (Scheibel et al., 1989; de la Torre, 2009). The model reproduces this general finding.

To show the effects of CVD in the model, a simulation is initiated with `CVD(1)` but all other input elements set to their normative levels. The presence of CVD in the model (Fig. 1) causes hypoxia (reduction in oxygen level) and ischemia (reduction in blood flow). In the model, hypoxia and ischemia occur at a level too low to cause cell death (apoptosis) by themselves, but occur at a level high enough to activate certain signaling proteins. Hypoxia activates HIF, which is a protein that up-regulates the transcription of the BACE gene and so increases the levels of BACEmRNA, BACE, and Abeta. Ischemia activates PERK, which is a protein kinase that phosphorylates and thereby activates eIF2, which is a protein that then up-regulates translation of BACEmRNA and so also increases the levels of BACE and Abeta (see Section 2.1). The level of Abeta reached as a consequence of CVD is high enough to close some of the pathological positive-feedback loops, which in turn close the others, leading to a build-up of Abeta that exceeds the threshold for AD in the model. Specifically, the command `rewrite [20] in ALZHEIMER: CVD(1)...`, where all other input elements are initially set to their normative levels, leads to `result State: CVD(1) hypoxia(1) HIF(1) ischemia(1) PERK(1) eIF2(1) BACEmRNA(7) BACE(8) Abeta(8) AD(1)...` (the other elements are omitted for clarity). The model illustrates how cerebrovascular disease that is not serious enough to cause cell death by itself can nevertheless lead to the development of AD in the model. This result demonstrates sensitive dependence.

The underlying reason for the sensitive dependence of AD on CVD in the model can be explored using logical model checking. The command `reduce in MC-ALZHEIMER: modelCheck (AM(CVD(1)...), ~(hasAD) U hasAPO)` checks whether apoptosis must occur before AD occurs in the model with CVD present, and the result is true. Similar checks show that cytokines must be activated before apoptosis occurs, so the same sequence of events leading to AD is followed in the model when CVD is present as when both IDE and NEP are absent. Together these results show that the model depends sensitively on CVD because it can initiate a cascade of threshold crossings and loop modifications that ultimately lead to a build-up of Abeta sufficient to cause AD in the model.

3.4. Local versus global

By flapping its wing in its local meadow, the proverbial butterfly changed the weather pattern on a global scale. Presumably, that butterfly was in just the right meadow, since butterflies flapping around in other meadows do not have such drastic effects on world climate. Put another way, butterflies that have the same local effects can have different global effects, depending on their location. Somewhat analogous phenomena are observed in the model. Due to model complexity, blocking the actions of factors that have the same local effects can have different global effects.

We can explore local versus global effects in the model by focusing on HIF and caspase3. HIF up-regulates transcription of the BACE gene and so increases the level of BACE (see Section 3.3). The protease caspase3 is activated by the process of apoptosis, after which it cleaves, and thereby inactivates, GGA3. GGA3 binds BACE and transports it to the lysosome for degradation (see Section 2.1 for references). Because precise quantitative information is unavailable, the absolute values of the influences of HIF on BACEmRNA, of BACEmRNA on BACE, of caspase3 on GGA3, and of GGA3 on BACE, are all set to 1. The HIF→BACEmRNA connection and the BACEmRNA→BACE connection are both positive, so HIF increases the BACE level by 1. The caspase3 ⊣ GGA3 connection and the GGA3 ⊣ BACE connection are both negative. Since two negative connections in series are equivalent to a positive connection, caspase3 also increases BACE by 1. Thus, both HIF and caspase3 increase the BACE level by 1. Because HIF and caspase3 have exactly the same local effect, it might be expected that blocking HIF would have the same global effect as blocking caspase3. Such is not the case.

The model includes the elements HIFblock and caspBlock, which block HIF and caspase3, respectively. More specifically, HIFblock and caspBlock ensure that HIF and caspase3, respectively, are at level 0 despite the presence of hypoxia, which would otherwise activate HIF, and of apoptosis, which would otherwise activate caspase3. If we acknowledge that local and global effects can be different in a complex system, then we can attempt to determine from inspection of the model diagram (Fig. 1) which blocker, HIFblock or caspBlock, would be more effective in reducing Abeta in the presence of CVD. We already know that CVD can initiate a cascade of increased Abeta production in the model that is sufficient to cause AD (see previous subsection). With this knowledge we might presume that blocking HIF would be more effective, because it would block the CVD→hypoxia→HIF pathway by which CVD triggers increased Abeta production in the model. Further inspection of the model diagram might dissuade us from this presumption.

On further inspection we note that the CVD→hypoxia→HIF pathway runs in parallel with the CVD→ischemia→PERK

pathway, and CVD can trigger increased Abeta production along either pathway (the HIF and PERK pathways both increase the level of BACE). Recognition of this parallel pathway might lead us to consider the relative benefits of blocking caspase3, which is an element in a pathological positive feedback loop through which Abeta can drive its own build-up. Blocking caspase3 would break this loop and should decrease the level of Abeta that is attainable in the presence of CVD. However, just as the HIF pathway is not the only forward pathway by which CVD can increase the level of Abeta, the caspase3 loop is not the only positive feedback loop by which Abeta can drive its own build-up. By itself, inspection of the model diagram leaves us unsure as to which blocker, HIFblock or caspBlock, would be more effective in reducing the level of Abeta in the presence of CVD. Simulation using the model can alleviate this uncertainty.

We can use rewriting commands to compare the effects of blocking HIF or caspase3 in the model. The command `rewrite [20] in ALZHEIMER: CVD(1) HIFblock(0) caspBlock(0)...` (where the other input elements are set to their normative levels) leads to `result State: Abeta(8) AD(1)...` (the other elements are omitted for clarity). As shown in Section 3.3, CVD increases Abeta from the normative level 4 to the pathological level 8. The effect of blocking caspase3 can be determined using `rewrite [20] in ALZHEIMER: CVD(1) HIFblock(0) caspBlock(1)...`, which leads to `result State: Abeta(7) AD(0)...`. This result shows that blocking caspase3 limits the rise of Abeta to level 7, which is just below the threshold for AD in the model. The effect of blocking HIF can be determined using `rewrite [20] in ALZHEIMER: CVD(1) HIFblock(1) caspBlock(0)...`, which leads to `result State: Abeta(5) AD(0)...`. This result shows that blocking HIF allows the Abeta level to rise by only 1 point, from 4 to 5, in the presence of CVD. Together these results show that blocking HIF has a much stronger global effect than blocking caspase3, even though both have the same local effect in the model. This finding underscores the fact that, in a complex system, the context in which an interaction takes place is as important as the interaction itself.

The results on HIFblock and caspBlock suggest that blocking HIF would be more effective than blocking caspase3 in reducing Abeta, in the presence of incipient CVD that is not, by itself, advanced enough to cause overt pathology. These results also provide an experimentally testable prediction from the model. Pharmacological blockers for both HIF and caspase3 exist: cilnidipine blocks HIF (Oda et al., 2009) while ifenprodil blocks caspase3 (Dave et al., 2003). Animals with HIF or caspase3 blocked could be subjected to brain hypoxia/ischemia via carotid occlusion (LeBlanc et al., 1994). The AD model predicts that the hypoxic/ischemic level of Abeta should be substantially lower with HIF blocked than with caspase3 blocked. Because the build-up of Abeta is considered to be the primary cause of AD (Hardy and Selkoe, 2002), the results of this test would have therapeutic relevance.

Considering that separately blocking HIF or caspase3 reduces the level of Abeta in the model in the presence of CVD, we should analyze the effect of blocking both together. The effect of blocking HIF and caspase3 together in the presence of CVD can be determined using the command `rewrite [20] in ALZHEIMER: CVD(1) HIFblock(1) caspBlock(1)...`, which leads to `result State: Abeta(5) AD(0)...`. This result shows that blocking HIF and caspase3 together is no more effective than blocking HIF alone in the presence of CVD. The reason for this can be appreciated by examining more broadly the result of blocking HIF alone, which is `result State: CVD(1) hypoxia(1) HIF(0) HIFblock(1) ischemia(1) PERK(1) eIF2(1) cytokines(0) OS(0) ERK(0) apoptosis(0) caspase3(0) caspBlock(0) Abeta(5) AD(0)...`. As expected, HIF is not activated despite hypoxia because HIFblock is present. With HIF blocked, CVD only raises Abeta to level 5, which is not enough to activate cytokines (or to activate ERK or to cause oxidative stress). Because apoptosis does not occur in the model until cytokines are activated (see Sections 3.2 and 3.3), apoptosis does not occur with CVD present but with HIF blocked. Because caspase3 is activated by apoptosis, caspase3 is not activated in the model with CVD present but with HIF blocked. Thus, if HIF is blocked in the presence of CVD, Abeta cannot be further reduced by also blocking caspase3 because caspase3 is not activated anyway.

Of course, this result depends sensitively on the values of the connections in the model. With HIF blocked, CVD still activates the PERK pathway. If the values of the connections along the PERK pathway were higher, it could tip the balance and initiate the Abeta cascade by itself, in which case blocking the HIF pathway would have no effect but blocking caspase3 would still have some effect on reducing the Abeta level. Recognition of the sensitivity of the model to the values of its connections is recognition of the need to test model predictions and to use the results, when necessary, to refine the model.

3.5. State-space search

Complex systems have large state spaces. The Maude search command is useful for searching a state space for states of particular interest. Because CVD is a trigger for the build-up of Abeta in the model, a state of particular interest would be that in which CVD is present, neither HIF nor caspase3 are blocked, and all other input elements are set at their normative levels, but AD does *not* occur. This search is initiated using the command `search [20] in ALZHEIMER: CVD(1) HIFblock(0) caspBlock(0) ... =>+ S:State AD(0)`, where `=>+` means search over one or more rewrites (rule executions) and `[20]` limits the state sequences to 20 rewrites. Any solutions to this search (in the form of sequences of states due to rule executions) would have to start with `CVD(1)` and arrive at a state with `AD(0)`. The search is conducted using the model as configuration for all of the simulations and analyses considered so far, that in which the declarations that activate cytokines, and that cause apoptosis and AD, are expressed as rules. This search finds two solutions. In the first solution, the rule that activates cytokines executes, but neither the rule that causes apoptosis nor the rule that causes AD executes. In the second solution, the rules that activate cytokines and cause apoptosis execute, but the rule that causes AD does not execute.

These are trivial solutions. Obviously, AD will not occur in the model if the rule that causes AD does not execute. Also, because AD will not occur until apoptosis occurs in the model, AD will not occur if the rule that causes apoptosis does not execute. These results show that searching for this state of interest, in which CVD is present and all other inputs are normative but AD is absent, does not produce useful results in the version of the AD model in which the declarations that activate cytokines, and that cause apoptosis and AD, are expressed as rules. It would be more useful to search for this state of interest in versions of the AD model in which other declarations are expressed as rules. In particular, it would be more useful to search the state spaces of versions of the AD model in which those declarations that represent branch points are expressed as rules. One branch point in the AD model involves the up-regulation of PS1 or PEN2 by the cjunJNK pathway. The other branch point concerns LRP, and whether it binds APP (LRPAPP), or binds apoE (LRPapoE), or binds Abeta at the BECs (BECLRP). We explore these two branch points in turn.

The AD model is modified so that the declarations that specify the regulation by cjunJKN of PS1 and PEN2 are expressed as rules (all other declarations are expressed as equations). The search is

again initiated using the command `search [20] in ALZHEIMER: CVD(1) HIFblock(0) caspBlock(0)... => + S:State AD(0).` In this case the search finds no solutions. The reason can be appreciated by considering the nature of the loops that regulate gammaSec in the model. Experimental work has indicated that the ERK and cjunJNK pathways have opposing effects on expression of the components of the gammaSec complex (Tamagno et al., 2009), and these effects are represented in the AD model. Specifically, ERK is activated and OS occurs at the same threshold level of Abeta in the model (Abeta level 5). ERK suppresses nicastrin from the normative level 2 to level 1, while OS, through the cjunJNK pathway, increases expression of PS1 and PEN2 from the normative level 1 to level 2. PS1, PEN2, and nicastrin are components of gammaSec, and the level of gammaSec can only be as high as the level of its lowest component. Because of the opposing effects of the ERK and cjunJNK pathways, the level of the lowest component of gammaSec always remains at 1. This can be appreciated by examining gammaSec regulation below and above the Abeta threshold for ERK and OS.

Below the Abeta threshold for ERK and OS, nicastrin is at level 2 but PS1 and PEN2 are at level 1, so gammaSec is also at level 1. Above the Abeta threshold for ERK and OS, nicastrin is at level 1 so gammaSec is again at level 1, whether or not the rules by which cjunJNK regulates PS1 and PEN2 execute and increase the PS1 and PEN2 levels to 2. To check this opposing effects mechanism we can use the command `reduce in MC-ALZHEIMER: modelCheck (AM(CVD(1)...), [] gSECeq1)` where `gSECeq1` is the property that gammaSec equals 1 and `[]` is the temporal logic operator for "always". The result of this check is true. By checking this property in the version of the AD model in which regulation by cjunJKN of PS1 and PEN2 is expressed using rules, the result shows that gammaSec is always at level 1 in the AD model, whether or not the Abeta threshold for ERK and OS is crossed, and whether or not the rules by which cjunJNK regulates PS1 or PEN2 execute. The gammaSec level would have to be reduced to 0 in the AD model in order to reduce the Abeta level but gammaSec remains at level 1, even in this version in which regulation by cjunJKN of PS1 and PEN2 is expressed using rules. Because of the opposing effects mechanism regulating gammaSec (Tamagno et al., 2009), the model version in which the cjunJNK to PS1 or PEN2 branch point is expressed using rules cannot reach a state in which CVD is present and all other inputs are normative but AD is absent. Searching the other branch point yields more useful results.

The AD model is again modified so that the declarations that determine the levels of LRPAPP, LRPapoE, and BECLRP are expressed as rules (all other declarations are expressed as equations). The search is again initiated using the command `search [20] in ALZHEIMER: CVD(1) HIFblock(0) caspBlock(0) ... => + S:State AD(0).` In this case the search finds one solution: `S:State -> CVD(1) hypoxia(1) HIF(1) cytokines(1) apoptosis(1) caspase3(1) LRPapoE(1) LRPAPP(0) BECLRP(1) RAGE(0) BACE(8) Abeta(7)....` Note that AD does not occur in this state (otherwise it would not be a solution), but HIF is activated, cytokines are activated, apoptosis has occurred, and caspase3 is activated. Using the rewrite command, as we did above, these events would have caused the Abeta level to exceed the threshold for AD. The search command found a state in which this did not occur. This state was reached through "unfair" application of the rewrite rules.

The rewrite command in Maude is "fair" in that all applicable rules get the chance to execute. When we use the rewrite command starting from a state that has CVD but is otherwise normal, we arrive at a state in which AD occurs. The search command can find states reachable through "unfair" application of the rules. That this occurred is apparent from the solution state listed above. Note that LRPapoE and BECLRP are present but LRPAPP is absent from the state found through search. The three declarations in module `ALZHEIMER` that make LRPAPP, LRPapoE, and BECLRP are the only three rules in this version of the AD model. A fair application of these rules makes LRPAPP, LRPapoE, and BECLRP provided that the genes for LRP and apoE are present, as they are in the normal case and in the CVD case at issue here. Unfair rule applications can lead to a state in which LRPapoE and BECLRP are present but LRPAPP is absent. In this state CVD is present but AD does not occur.

LRP, itself a protein, is involved in the trafficking of other proteins and peptides such as APP and Abeta. LRP can bind APP (LRPAPP) and make it more available to BACE and gammaSec, so LRPAPP increases the Abeta level. Apolipoprotein E (apoE) is another trafficking protein, and it can bind several molecules of Abeta (see Section 2.1). LRP can then bind apoE forming the LRPapoE complex, endocytosis of which leads to lysosomal degradation of the Abeta bound to apoE. LRP can also bind Abeta directly, and when it does it can transcytose Abeta over the brain epithelial cells (BECs) that compose the blood–brain barrier, thereby exporting Abeta from the brain. Thus LRPAPP increases the Abeta level, while LRPapoE and BECLRP decrease the level of Abeta in the brain.

Search reaches a state in which the rules that make LRPapoE and BECLRP executed but in which the rule that makes LRPAPP did not execute. Formation of LRPapoE and BECLRP combined with failure to form LRPAPP results in a decrease in the Abeta level to 7, which is just enough to prevent the occurrence of AD in the model. This solution is not immediately apparent from inspection of the model diagram (see Fig. 1), but was revealed through a search command issued in Maude. This result can be used to derive the prediction that compounds that selectively prevent binding of LRP to APP but do not prevent binding of LRP to apoE or to Abeta directly should reduce the level of Abeta in the presence of incipient CVD.

4. Discussion

Reasoning effectively about the pathogenesis of AD is difficult because of the sheer complexity of the disorder. By representing the data on AD pathophysiology as an executable mathematical theory in Maude, inferences can be drawn from the data that might remain hidden without the aid of the computational model. Because the model represents many interactions it can also help in the interpretation of data. One example concerns the influence of LRP on Abeta levels.

4.1. Data interpretation and the role of LRP

Previous and ongoing work shows that LRP can bind APP and make it more available to BACE and gammaSec, thereby increasing the production of Abeta (Ulery et al., 2000; Yoon et al., 2007; Lakshmana et al., 2008). Recent work by Minopoli et al. (2007) demonstrates that phosphorylation of LRP by a tyrosine kinase can further increase the level of Abeta. This seems to suggest that phosphorylation of LRP increases its affinity for APP, and so increases the level at which it makes APP available to BACE and gammaSec. This suggestions appears to be contradicted by the finding (Pietrzik et al., 2002) that cells deficient in LRP have higher levels of Abeta compared to normal cells (if LRP increases Abeta, and phosphorylated LRP further increases Abeta, then one would expect that LRP deficiency should decrease Abeta). This led Minopoli et al. (2007) to hypothesize that phosphorylation of LRP actually decreases its affinity for APP, but this hypothesis does not take into account the interactions between LRP and apoE. Because

apoE binds Abeta oligomers, and because LRP binds the apoE/oligomer complex and traffics it to the lysosome for degradation (Strittmatter et al., 1993; Manelli et al., 2004), LRP actually removes more Abeta than it helps to produce in normal cells. This dual role of LRP in cells is represented in the model, and it provides a possible clarification of these findings.

In the model, LRP is either bound to APP (LRPAPP), bound to apoE (LRPapoE), or bound to Abeta directly at the BECs (BECLRP) (the BECs compose the blood–brain barrier). The experiments of Minopoli et al. (2007) were done in cell culture so the simulated actions of LRP at the blood–brain barrier are not directly relevant to them. Because LRP binds a single molecule of APP, but apoE binds Abeta oligomers, LRPAPP increases Abeta by 1 while LRPapoE decreases Abeta by 2 in the model (see Section 2.2). Therefore, the effect of LRPAPP and LRPapoE together is to reduce the Abeta level by 1 in the normative state. An increase in the affinity of phosphorylated LRP for APP can be simulated in the model by increasing the positive influence of LRP on Abeta to 2. Now the effects of LRPAPP and LRPapoE cancel, so the net effect of both is zero, which means that the simulated phosphorylation of LRP should increase the Abeta level in the model. It does, and this is consistent with the findings of Minopoli et al. (2007). LRP deficiency in the model can be simulated by removing the LRP gene. Obviously, with LRP absent the net effect of LRPAPP and LRPapoE is also zero, which similarly increases the Abeta level, and this is again consistent with the data (Minopoli et al., 2007; Pietrzik et al., 2002). Thus, the initial, more parsimonious assumption, that phosphorylation of LRP increases its affinity for APP, is consistent with available data. The model can resolve this seeming paradox because it brings together enough of the relevant biology.

In the full model, removing LRP (by removing the LRPgene) not only eliminates LRPAPP and LRPapoE but BECLRP as well. Since BECLRP opposes RAGE, and RAGE increases the Abeta level (by importing Abeta from the periphery into the brain), elimination of BECLRP in the model further increases the Abeta level over that due to elimination of LRPAPP and LRPapoE. Thus, the net effect of LRP in the model (i.e. the combined effects of LRPAPP, LRPapoE, and BECLRP) is to decrease Abeta. Unfortunately, there are no data as yet with which to compare this modeling result because the LRP knockout is embryonic lethal, but conditional LRP-deficient mice may provide relevant data in future (Harris-White and Frautschy, 2005).

As mentioned above, LRP contributes to the cellular degradation of Abeta by binding and endocytosing apoE after apoE has bound Abeta oligomers (Strittmatter et al., 1993; Manelli et al., 2004). The three most common apoE isoforms are apoE2, apoE3, and apoE4, and the apoE4 isoform is associated with increased risk of AD (Saunders and Apolipoprotien, 2000). Of the three isoforms, the apoE4 isoform is the least effective in promoting Abeta endocytosis (Yang et al., 1999). The reduced effectiveness of apoE4 compared with the other two common apoE isoforms can be simulated in the model simply by reducing from 2 to 1 the absolute value of the negative connection from apoE to Abeta. Doing so, as expected, increases the level of Abeta in the model.

4.2. Reasoning and the potential for multi-drug therapy

The examples in Section 4.1 illustrate how the data-driven AD model can represent and interpret existing findings on AD pathophysiology. The model also facilitates the kind of reasoning that could lead to therapeutically useful outcomes. For example, ERK is known to negatively modulate gammaSec by phosphorylating nicastrin when Abeta levels are high, but the mechanism by which Abeta activates ERK is not known (Kim et al., 2006). One possibility is that Abeta activates ERK via the rapidly accelerated fibrosarcoma (RAF)–ERK pathway, and it is also possible, due to genetic variation in RAF, that the RAF–ERK pathway is blocked in some phenotypes (Wan et al., 2004). Inability to activate ERK has interesting effects in the model.

The ERK and cjunJNK pathways balance each other in the model, so that the level of gammaSec stays constant even after the Abeta level rises above the threshold for activation of both pathways (see Section 3.5). This model feature is consistent with experimental findings (Tamagno et al., 2009). The constancy of the gammaSec level is one reason why blocking HIF is sufficient to restrict Abeta to level 5 in the presence of CVD. In this case, also blocking caspase3 provides no further reduction in Abeta (see Section 3.4). However, blocking ERK activation allows the level of gammaSec to rise, and this leads to AD in the model in the presence of CVD, even if HIF is blocked. In this case, also blocking caspase3 can reduce the level of Abeta to 7, which is below the AD threshold in the model. Thus, in the presence of CVD, blocking caspase3 in addition to blocking HIF might be useless if ERK phosphorylates nicastrin, but useful in a phenotype in which the ERK pathway is blocked.

Pharmacologically, the most direct ways to reduce the level of Abeta would be to block the enzymes that produce it from APP, namely BACE and gammaSec (Citron, 2010). BACE is highly specific, which makes it an attractive target for drug development (Vassar et al., 2009; Cole and Vassar, 2007). Unfortunately, BACE has a very large active site, and finding a compound big enough to block BACE but small enough to cross the blood–brain barrier has been difficult (Arun et al., 2008). While gammaSec is easier to block, doing so has serious side effects. Because Notch is also a gammaSec substrate, blocking gammaSec disrupts Notch signaling and this causes immunological and gastrointestinal disturbances even in adults (Imbimbo, 2008). Some compounds, including certain NSAIDs, have been found to modulate gammaSec activity by reducing APP cleavage but sparing Notch cleavage (De Strooper et al., 2010). These compounds would reduce Abeta without causing side effects, but the question remains as to whether they could reduce Abeta enough to prevent AD.

Different drugs can have the same desired effect but different side effects. One possible strategy for the treatment of AD involves multi-drug therapy in which each drug is administered at a dosage low enough that its side effects are minimal but high enough that, in combination with the other drugs, it produces a clinically significant reduction in Abeta. One possible approach would be to administer a partially effective BACE inhibitor and a gammaSec modulator together. The model suggests other possible approaches to multi-drug therapy in AD.

The AD model in its present form demonstrates how incipient cerebrovascular disease could set off a cascade of events that would cause Abeta to rise to the level at which AD results. The model further suggests that blocking HIF under these circumstances could reduce Abeta levels, but not bring them all the way down to the normative level (see Section 3.4). The failure of HIFblock to bring Abeta down to the normative level is due to the parallelism of the pathway by which CVD influences Abeta metabolism in the model. HIFblock only blocks one side—it leaves the PERK side open. Additional simulations show that blocking both HIF and PERK can reduce Abeta down to the normative level in the presence of CVD. In case a specific pharmacological blocker for PERK cannot be found, the model offers an alternative. NSAIDs are known to up-regulate PPAR, while PPAR down-regulates BACEmRNA and thereby down-regulates BACE and Abeta. In the model, the combination of HIFblock and NSAIDs is able to reduce Abeta all the way down to the normative level in the presence of CVD. It would be of interest experimentally to test the effects of a HIF blocker combined with NSAIDs on the Abeta level in animals. These results demonstrate the usefulness of data-driven

computer models in the development of combination drug therapies for complex disorders.

4.3. Future work and model extensions

The AD model is readily expandable to include more of the molecular species involved in AD and to represent various pathological processes in greater detail. For example, the CVD that may trigger the cascade that results in Abeta accumulation in AD may itself result from dysregulation of the mesenchyme homeobox 2 (MEOX2) gene, or the genes for serum response factor (SRF) and myocardium (MYOCD), which encode proteins that regulate the viability and contractility of cerebral blood vessels (Wu et al., 2005; Chow et al., 2007; Bell et al., 2009). The model represents Abeta regulation as it transitions from the normative to the pathological state, rather than the state of advanced AD pathology, but it could be expanded to account also for chronic conditions. For example, chronically elevated levels of Abeta can cause capillary occlusion and cerebral blood flow disturbances (Thal et al., 2008), which may in turn lead to dysregulation of MEOX2, SRF, and MYOCD that further reduces blood flow (Wu et al., 2005; Chow et al., 2007; Bell et al., 2009; Xia et al., 2011). Thus, in the chronic state, there may be an encompassing feedback loop from Abeta back to CVD. This and other pathways could be added to the model to account for chronic conditions.

Table 1
The elements of the data-driven model of Alzheimer Disease.

ELEMENT	WHAT IT IS	WHAT IT DOES
Abeta	Beta-amyloid	Causes OS and apoptosis, and acts as a signaling molecule
AD	Alzheimer Disease	Kills the brain
apoE	Apolipoprotein E	Binds several molecules of Abeta
apoEgene	apoE gene	Codes for apoE
Apoptosis	The process of cell death	Kills individual neurons and other cells
APP	Amyloid precursor protein	Protein source of Abeta
APPgene	APP gene	Codes for APP
BACE	Beta secretase (BACE1)	Cleaves APP, leaving C-terminal fragment (C99)
BACEASRNA	BACE antisense RNA	Stabilizes BACEmRNA
BACEASgene	BACEASRNA gene	Codes for BACEASRNA
BACEgene	BACE gene	Codes for BACE
BACEmigene	BACEmiRNA gene	Codes for BACEmiRNA
BACEmiRNA	BACE micro-interfering RNA	Interferes with translation of BACEmRNA
BACEmRNA	BACE messenger RNA	Provides message for BACE translation
BECLRP	LRP acting at the brain endothelial cells that form the blood–brain barrier	LRP can bind Abeta directly and export it from the brain over brain endothelial cells (BECs)
caspase3	Caspase 3	Cleaves and inactivates GGA3
caspBlock	Blocker of caspase3	Blocks action of caspase3 (e.g. ifenprodil)
cjunJNK	c-Jun N-terminal kinase pathway	Enhances expression of PS1 and PEN2
CVD	Cerebrovascular disease	Causes hypoxia and ischemia in the brain
cytokines	Immunological proteins	Suppress expression of PPAR
eIF2	Eukaryotic translation initiation factor-2-alpha	Enhances expression of BACE when phosphorylated
ERK	Extracellular signal-regulated kinase	Negatively modulates nicastrin by phosphorylating it
gammaSec	Gamma-secretase	Cleaves C99 producing Abeta
GGA3	Golgi-localized gamma-ear-containing ADP-ribosylation factor-binding protein	Binds BACE and transports it to lysosmes where it is degraded
GGA3gene	GGA3 gene	Codes for GGA3
hepSul	Heparan sulfate	Inhibits binding of BACE and APP
HIF	Hypoxia-inducible factor-1-alpha	Enhances expression of BACEmRNA
HIFblock	HIF block	Blocks action of HIF (e.g. cilnidipine)
Hypoxia	Lack of oxygen	Damages or kills neurons and other cells
IDE	Insulin degrading enzyme	Degrades Abeta
Ischemia	Lack of blood flow	Damages or kills neurons and other cells
LRP	Lipoprotein receptor-related protein (LRP1 specifically)	Binds APP, apoE, or Abeta
LRPapoE	LRP–apoE complex	Transports Abeta to lysosomes for degredation
LRPAPP	LRP bound to APP	LRP binds APP and transports it to lipid rafts where it is made available to BACE and gammaSec
LRPgene	LRP gene	Codes for LRP
NEP	Neprilysin	Degrades Abeta
nicastrin	Nicastrin	Component of gammaSec
NICgene	Nicastrin gene	Codes for nicastrin
NSAID	Non-steroidal anti-inflammatory drugs	Enhances expression of PPAR
OS	Oxidative stress	Damages or kills neurons and other cells
PEN2	Presenilin enhancer-2	Component of gammaSec
PEN2gene	PEN2 gene	Codes for PEN2
PERK	Pancreatic endoplasmic reticulum eIF2alpha kinase	Phosphorylates and thereby activates eIF2
PPAR	Peroxisome proliferator-activated receptor gamma	Suppresses expression of BACEmRNA, either directly or indirectly
PPARgene	PPAR gene	Codes for PPAR (PPAR gamma)
PS1	presinilin 1	Component of gammaSec
PS1gene	PS1 gene	Codes for PS1
RAGE	Receptor for advanced glycation end products	Imports Abeta from the peripheral circulation into the brain
RAP	Receptor-associated protein	Binds Abeta and transports it to lysosomes for degradation
RTN3	Reticulon-3	Inhibits binding of BACE and APP
seladin1	Selective Alzheimer Disease indicator 1	Decreases caspase3 activity under apoptotic conditions
SNX6	Sorting nexin-6	Binds Abeta and transports it to lysosomes for degredation

The first column lists the element name as an abbreviation of a condition or molecule. The second column spells out the name of the element, and the third column describes its role in the model.

The model can also be expanded to include the various competing hypothesis concerning AD etiology. The model is based mostly on the amyloid hypothesis (Hardy and Selkoe, 2002) and partly on the hypothesis that incipient CVD can trigger AD (Scheibel et al., 1989; de la Torre, 2009). Other hypotheses assign the cause of AD to diabetes (Sims-Robinson et al., 2010), inflammation (Lue et al., 2010), or neurofibrillary degeneration (Iqbal et al., 2010), but all authors stress that the various theories are not mutually exclusive. In representing the findings on which these various views are based, future versions of the model may help to reconcile them.

5. Conclusions

Alzheimer Disease is a complex neurological disease process with high prevalence that poses a serious societal threat. This paper illustrates that data-driven computer models are a useful adjunct to experiment in AD research. Data-driven models are based directly on data derived from experiments. As such, they provide insights of immediate relevance to the experimentalists who gathered the data, and they generate predictions that, in most cases, are readily testable using small modifications of the same experimental procedures that were used to gather the data on which the model is based. A data-driven model is expanded using additional findings and refined according to the results of experiments that test its predictions. The result is a model that should represent much of what is known concerning a phenomenon, and that can be used to provide new insights and make well-founded predictions that take more of the data into account than would be possible without the model. This approach is well suited to complex biological phenomena such as neurological disease processes including AD. This paper also shows that Maude, which is a mathematical modeling language designed to represent and analyze models based on systems of equations and rules, provides an ideal tool for data-driven AD modeling.

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