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EDITORIAL

Multiscale modeling for drug discovery in brain disease

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Introduction

In this special issue on *Computational Models of Brain Disorders*, we introduce readers to a set of studies using *multiscale models* (MSMs) – computer simulations of brain areas used to understand diseases and disorders of the brain. Many of the MSMs in this issue are *mechanistic*. This means that they describe a high level in terms of what is going on at one or more lower levels. For example, the dynamics in a neuronal network can be explained in terms of cell or even of ion channel dynamics. Mechanistic MSM is directly explanatory. Mechanistic modeling can be contrasted with phenomenological modeling. Phenomenological models describe the external manifestations of observed data without reference to underlying mechanism. This approach follows the physics tradition of describing phenomena as sets of basic 'natural laws,' where either no underlying mechanism is known, or where one putatively describes an ur-reality so that no underlying mechanism exists.

MSM can play a number of roles in the clinical sphere, several related to drug development. 1. Models can be directly used to predict the effects of specific medications which can then be used in animal models to confirm efficacy before trying in a formal clinical trial. 2. Models can be used for *precision medicine*, that is, directed at identified subpopulations

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who have a particular subtype of disease, generally based on their genetic inheritance. 3. Models can be used for *personalized medicine*, where a simulation models an individual patient. This approach is already being used for epilepsy surgery, where every procedure is necessarily personalized due to the differences in each patients brain and seizure focus [1–4]. 4. Models can assist in our general understanding of pathophysiology. Understanding provides opportunities for judgement-based medicine, where drugs are proposed, and often used off-label, based on intuitive approaches without additional formal modeling. 5. Models can identify biomarkers. This is particularly important for diseases, such as Alzheimer, where the current lack of biomarkers makes it impossible to make a definitive diagnosis during life. Proper diagnosis is of course a prerequisite for proper treatment. 6. Models can be used to identify and treat disease subtypes – some brain diseases are not unitary but represent a final common pathway of expression for several different pathologies with different causes (Alzheimer here again an example). Different subtypes may benefit from different treatments. 7. Models can be used to identify the course of pathophysiology to permit *staging* of disease. In many cases, as with cancers, treatments will be different depending upon disease stage. 8. Models can assist in determining prognosis, an important clinical determination that assists in identifying the proper type of drug to be administered or discovered. Given the complexities of expression, a number of brain disease have *formes frustes* (forms of the disease with reduced or minimal symptomatology) which may or may not merit treatment.

The authors in this issue have used their models to investigate a variety of the brain diseases treated by neurology, neurosurgery, psychiatry, psychiatry, internal medicine and other specialties including Alzheimer, Parkinson, Huntington, ALS, epilepsy, depression, dystonia, and essential tremor. In many of these studies, an MSM is first used to replicate the dynamics of brain activity, and then modified to replicate the pathophysiological dynamics of the particular disorder. The pathophysiological simulations are explored to find pharmacologically related parameter changes that could suggest drug treatments to transform pathophysiological to physiological dynamics. In this way, the authors use the models to predict pharmacological or electrical-stimulation treatments which could be tested in animals for subsequent clinical use. In the following, we loosely group the contributions based on spatial scales used, on disorders discussed, or on proposed methodological advances.

Electrophysiology has been the focus of both traditional neurophysiology and computational neuroscience for decades. Only recently have these fields begun to appreciate the importance of extending their studies into the vast realms of chemophysiology associated with 'omics – particularly transcriptomics and proteomics. Incorporation of chemophysiology allows more detailed explorations of pharmacological treatments for brain disorders through specification of molecular signaling cascades that can be altered with pharmacological treatment. Several papers in this issue highlight recent developments in multiphysics simulation (multiphysics implies the combining of models representing different types of physical phenomenology) which allow this combining of chemophysiology and electrophysiology. In this issue, Anderson and Vadigepalli [5] use a multiphysics model to investigate inflammatory regulatory network dynamics in central nervous system disorders. Anwar [6] investigates the role of the ubiquitous second-messenger, calcium, and how its dysregulation can lead to neurodegenerative disorders. Extending this methodology from intracellular to extracellular diffusion, Newton and Lytton [7] investigate how spread of extracellular toxins produces a spreading depression that can contribute to pathological effects in epilepsy, migraine and stroke. Neymotin et al. [8] identify sources of hyperexcitability in a microcircuit model of motor cortex which includes detailed intracellular molecular dynamics, developing a methodology for classifying sets of parameters relating to disease which could be used to predict multitarget therapeutics.

Several models focus on the neuronal network level, including Cutsuridis and Moustafa [9] on Alzheimer disease, and Lytton [10] on epilepsy. Note that MSM techniques of neuronal network modeling are entirely different from *artificial neural networks*, a class of machine learning techniques that can also be used in drug discovery. Machine learning techniques are used in clinical analysis of *big data* including the big data associated with drug trials.

A pair of papers utilize computer models to determine how electrical treatments are used to treat movement disorders, with Holt and Netoff [11] looking at Parkinson, and Lee et al. [12] at essential tremor. Both of these diseases produce tremor, and both can be treated with electrical stimulation of telencephalic nuclei using deep brain stimulation (DBS). Modeling can help us better understand why DBS works for some patients and not for others, and how to best use DBS as a complement to traditional drug therapies.

At the highest spatial scale, a pair of reviews describe modeling of interactions across brain areas. Bernard and Jirsa [1] describe The Virtual Brain (TVB; thevirtualbrain.org), a simulation tool that can simulate the entire brain as a set of interconnected neural mass models. Arle and Carlson [13] demonstrate a multi-area circuit model in their Universal Neural Circuitry simulator (UNCuS) to look at depressive disorder.

In two papers, the authors describe phenomenological computational methods to evaluate pharmacological agents for neurological diseases without use of explicit simulation. Anastasio [14] introduces *process algebra*, a computer technique that is widely used to analyze complex computational systems, here used for computational neurology. Sirci et al.

[15] describe the use of network (graph) theory to identify similarities and differences between different pharmacological agents. In this type of study, each drug is a *node*, and *edges* between drugs represent chemical and transcriptional-based interactions that describe drug properties.

Overall, the series of papers in this issue represents an alternative to the classical medical approach which dates back to *Koch's postulates* of more than a century ago. Koch set forth rules for identifying an agent considered as sole cause for a given infectious disease. The early history of antimicrobial drug discovery involved the search for the 'magic bullet' drug to eradicate that agent. This thinking extended into the modern era, with many hoping and expecting that the human genome would reveal single gene causes of disease that could then eventually be fixed with genetic engineering. Instead, genetic analysis of human disease revealed polygenic risk factors (rather than causes) that combine with environmental risk factors, including infections, to produce disease. Multiscale modeling is now emerging to begin to master this complexity by disentangling how risks and causes combine in complex systems to produce disease, and how these diseases could be prevented or ameliorated, perhaps by multi-staged, multi-target, polypharmaceutical techniques.

Conflict of interest

The authors have no conflict of interest to declare.

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Computational Models of Neurological Disorder

Virtual Brain for neurological disease modeling

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Neurological disorders are often characterized by alterations in multiple brain regions; i.e. they should be studied at the organ level. Neuroimaging techniques allow extracting information at the whole brain level, but a conceptual framework is lacking to interpret neuroimaging data. The Virtual Brain (for humans) and its extension The Virtual Mouse Brain (for rodents) can provide such a framework. These platforms enable the virtualization of individual brains based on Diffusion-weighted Tensor Imaging, thus allowing studying whole brain dynamics *in silico*. In addition to the analysis structure/function relationships, the models can be used to generate testable predictions, including in the clinic to improve patient's care.

Introduction

Neurological disorders represent a large cost to society, $1.5 trillion/year, nearly 9% of the gross domestic product (World Health Organization (2006)). Most of brain disorders, such as migraine, epilepsies, Alzheimer's disease, Parkinson's disease, and major depression remain poorly treated. Their progression can be slowed down; but it is often just a temporary relief with a large inter-patient variability. For instance many patients cannot be treated with existing drugs, e.g. 30% for patients with epilepsy. These facts underscore the necessity to better understand patient-specific mechanisms underlying brain disorders. Historically personalized medicine uses heavily genetic information, but finds more and more response on the system level. Structural and functional neuroimaging play a key role and have contributed diagnostic tools, e.g. such as presurgical evaluation of epilepsy. One solution to this issue is to link the interpretation of neuroimaging signals to personalized computational brain models, which we discuss in the following.

Brain disease modeling

Two different mechanistic modeling approaches can be distinguished according to the desired effect, i.e. whether one looks for a preventive or a curative treatment. The preventive approach requires identifying predictive biomarkers and the causal factors responsible for the transformation of a ''healthy'' network into a pathological one. The curative approach includes repairing the system, or at least controlling the symptoms. The mechanisms that need to be targeted for preventive and curative approaches are not necessarily identical. Co-morbidities (depression, cognitive deficits etc.) are also an integral part of the problem. For example, depression is associated with most, if not all, neurological disorders [1–3]. Likewise, the mechanisms of co-morbidities are not necessarily identical to those of the underlying pathology, which implies the necessity to identify multiple therapeutic

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targets. Finally, most, if not all, neurological disorders involve multiple interconnected brain regions defining a network problem. The problem should be grasped at least at the organ level, i.e. the whole brain. Even a dysfunctional small region may have large-scale repercussions, perturbing whole brain functions. Brain imaging techniques have provided a wealth of information on the reorganizations taking place in patients with neurological disorders. In particular, most pathological states are associated with metabolic dysfunction, a modified connectome, and altered whole brain dynamics, which may even be predictive of the evolution of the pathologies [4–7]. However, these findings are difficult to decipher, since we lack a conceptual framework to interpret them. In particular, it is difficult to determine whether these alterations are causally related to the pathology/symptoms or they are just biomarkers, or a consequence of the pathological process.

Computational models can provide such a conceptual framework, and provide us with ways to explore causality *in silico* and generate testable hypotheses. One can distinguish several levels of modeling. At one end of the spectrum, there is the Human Brain Project (https://www.humanbrainproject.eu/), which aims to construct neuronal networks taking a constructive bottom-up approach involving a large degree of subcellular and cellular detail. Since all biophysical parameters are included, such type of modeling offers mechanistic insights and the possibility to create simulation platforms for systematic discovery of novel therapeutic targets. In the long run, this approach is the way to go, however, it will take time, computational resources and requires the development of novel super computer technologies. At the other end of the spectrum, one finds The Virtual Brain (TVB), a top-down approach allowing simulating whole brain dynamics today [8]. This article focuses on TVB and its possible use to further our knowledge of neurological disorders.

The Virtual Brain

The Virtual Brain (TVB) is a large-scale brain network model comprising a connectivity matrix between cortical and subcortical areas and network nodes representing brain areas. TVB connectivity for primates is typically derived from Diffusion-weighted Tensor Imaging (DTI), a fairly recent non-invasive technology allowing the reconstruction of the myelinated white matter fibers. Brain areas are modeled using neural population models of varying degrees of sophistication. The computational models simulate the neural activity in brain regions and allow computing secondary signals commonly used in human brain imaging, including surface electroencephalography (EEG), magnetoencephalography (MEG), intracranial EEG (iEEG), stereotactic EEG (sEEG), and functional Magnetic Resonace Imaging (fMRI) signals using biologically realistic head models. This unique approach allows creating a brain network constrained by subject specific structural imaging data and computing activity, which can be directly compared with empirical functional data. One key feature unique to large-scale brain networks is the consideration of spatially distributed signal transmission delays due to finite speeds [9,10], which render the network dynamics complex, even if the isolated network node dynamics remains simple. Obviously this approach does not have the richness of detailed bottom-up approaches, but it can exploit the predictive power of the network dynamics and explore questions, which are due to network alterations. It is this idea that is key to The Virtual Brain approach and has been first formulated seventeen years ago in Refs. [11,12]. Pathologies, for instance, linked to widely distributed network dysfunctions are natural candidates for Virtual Brain based modeling including epilepsy, schizophrenia, multiple sclerosis and stroke. In epilepsy, mechanisms of seizure propagation can be studied in a patient's brain with the objective to tailor therapeutic and surgical interventions specifically to the patient's own brain connectivity. For stroke patients, lesion masks can be directly transferred to the Virtual Brain to allow simulations of brain activity based on the patient's altered connectivity.

To aid in the exploration of network-based phenomena in neuromodeling of brain function and associated pathologies, Jirsa *et al.* [13] proposed the creation of a neuroinformatics platform, which became The Virtual Brain (TVB). TVB is a free open source neuroinformatics tool written in Python. A first prototype was launched in October 2012 and is regularly maintained and updated since then [8]. TVB provides the possibility to feed computational neuronal network models with information about structural and functional imaging data including population (sEEG/EEG/MEG) activity, spatially highly resolved whole brain metabolic/vascular signals (fMRI) and global measures of neuronal connections (DTI) – for intact as well as pathologically altered connectivity. TVB is model agnostic and offers a wide range of neural population models to be used as network nodes. The software infrastructure of the Virtual Brain is composed of a functional core running the large-scale brain simulations independently or in batch mode, a web based interface to access the simulator, as well as a command line interface to develop more extensive applications. All simulations may be performed on workstations and laptops, as well as on high-performance clusters (HPCs). Manipulations of network parameters within the Virtual Brain allow researchers and clinicians to test the effects of experimental paradigms, interventions (such as stimulation and surgery) and therapeutic strategies (such as pharmaceutical interventions targeting local areas). The computational environment allows the user to visualize the simulated data in 2D and 3D and perform data analyses in the same way as commonly performed with empirical data.

The mathematical and technical details of TVB are provided in Refs [14,15]. A semi-analytically treatable example is found in Ref. [16].

In TVB clinics we apply concepts, theory and modeling derived from the perspective of large-scale networks to clinical questions in epilepsy and neurodegenerative diseases. Clinical observations of patients with brain injury reveal a highly variable relationship between the structural abnormalities (lesions, etc) and the functional deficit. That is, while some classic 'deficit syndromes' do exist, there is also large variation among patients. Furthermore, there is a highly variable pattern of cognitive deficits within a single patient; language, memory and planning may be affected quite differently. Lesions involving large areas of the brain may result in only subtle deficits, whereas less extensive lesions may profoundly affect a number of cognitive domains. Distributed pathology – such as occurs in disease of the small cerebral vessels or dementia – may only cause noticeable deficits when sufficiently taxing cognitive effort is required. The basis for such variation is poorly understood. The exploration of the underlying biological process and the identification and development of novel therapies or targets necessitates dynamic network perspective and a more profound understanding of the relations and causal links between the heterogeneous factors.

The key-defining feature of TVB is that it can be personalized, using neuroimaging data from a single person to essentially make their brain The Virtual Brain. An exciting clinical potential is that therapeutic interventions could be assessed in a patient-specific Virtual Brain first to help converge on pathways that are most likely to have the best outcome. Following such assessment, novel multi-factorial biomarkers can be developed and optimized that allow an efficient tracking of the patient's recovery. A typical workflow in TVB Clinics is shown in Fig. 1.

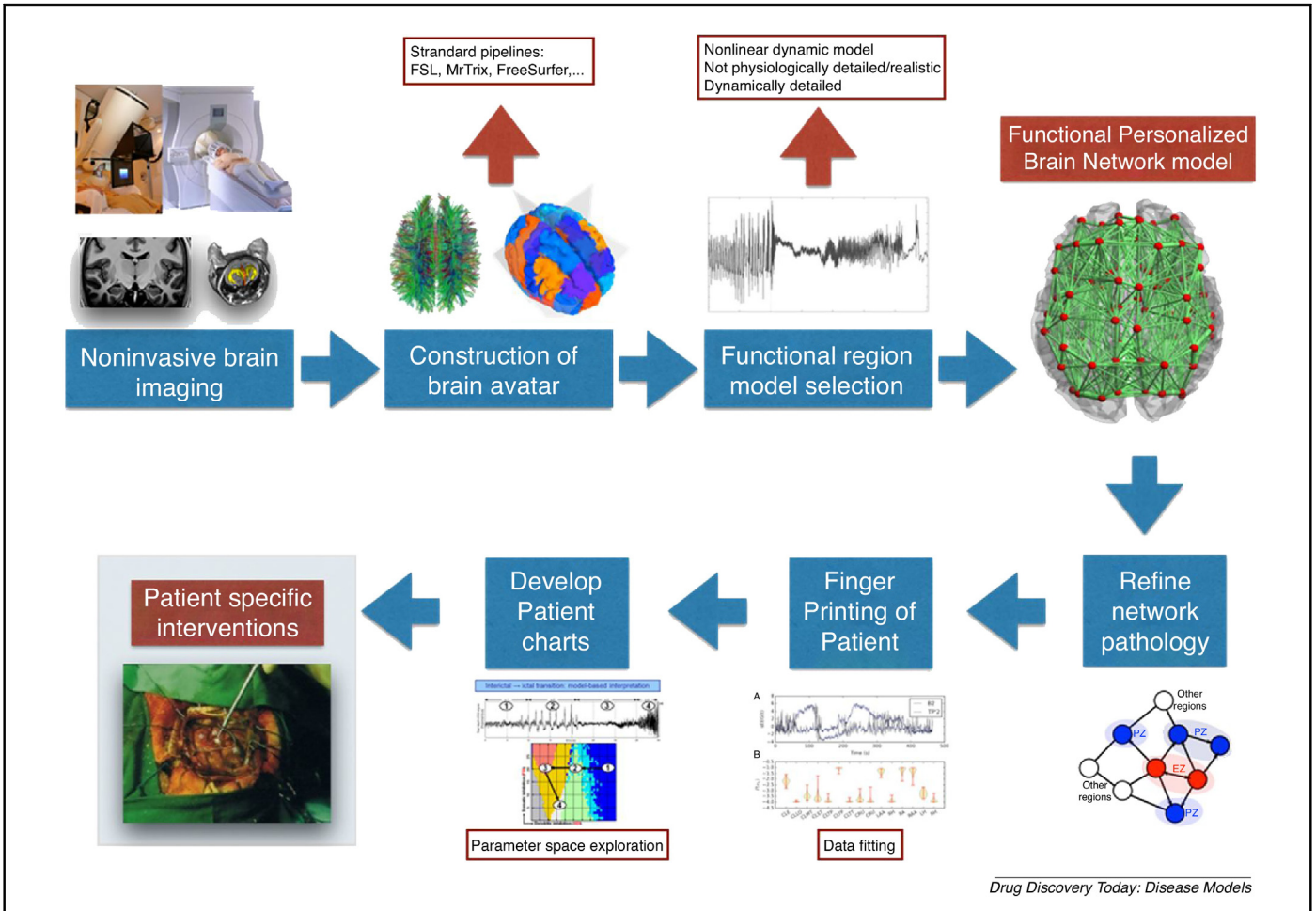

Figure 1. Work flow in The Virtual Brain for clinical applications. Patients undergo non-invasive brain imaging (MRI, DTI). Based on these images a virtual brain avatar is created, identifying a network skeleton in 3D space. Then a neural population or mean field model is selected for each brain region, appropriate to address the brain region dynamics. Put together, mathematical models and avatar define the patient's virtual brain. Additional structural knowledge, such as a clinical hypothesis on the location of the Epileptogenic Zone or lesion, allows refining the network pathology. Subsequent simulations allow fitting the virtual brain model against functional brain imaging date (finger printing) and explore the consequences of patient specific network parameter manipulations (patient charts). Interpretation of fingerprint and patient chart provides additional information to the clinician in the decision-making on a patient's treatment.

Modeling serves as a powerful research tool to interpret data and develop theory. The individualization of brain models allows us to create one model per person and systematically assess the modeled parameters that relate to individual differences. Generative models are the only means to establish causality of aetiopathogenetic pathways and systematically explore novel therapeutic directions. In the best case, generative models include mechanistic multiscale descriptions of brain network systems that can be personalized for a patient and generate biologically realistic time resolved data mimicking the empirical data sets obtained from invasive and non-invasive human brain imaging. A complete multiscale (spatial and temporal) description of the human brain is still decades away, even though massive European investments (e.g. the FET flagship Human Brain Project) are prioritizing this development. State of the art today are data mining approaches informed by prior knowledge derived from meta analysis, literature mining and semantic analysis procedures, which identify links in heterogeneous data leading to subspaces highly associated with certain phenotypes, but are by construction incapable of predicting modalities outside of the parameter space, for which data is available. In TVB clinics we aim to combine the predictive power of generative brain network models with the multiscale parameter structures and phenomenological correlations obtained from data mining taking advantage of the respective strengths of both approaches. Rather than mechanistically modeling every level of organization (from the molecular to the non-invasive brain imaging level), we inform the mechanistic brain network models by the outcomes of sophisticated data mining, which spans the links across heterogeneous data types and thus traverses the scales of organization. This novel hybrid concept will accelerate our current way of thinking in multiscale modeling and develop new technologies to identify patterns of alteration across different levels of biological organization, suggesting new diagnostic indicators and drug targets, facilitating the selection of subjects for clinical trials, providing the data required for disease modeling and simulation, and facilitating the translation of knowledge about the brain from the laboratory to the clinic.

The Virtual Mouse Brain (TVMB)

There is a multiplicity of experimental models of neurological disorders. These models have been designed to explore mechanisms and therapeutic solutions. One can distinguish genetic from induced models. The most relevant genetic models are those for which a mutation found in human families is directly introduced in the rodent genome. Induced models mostly involve lesions (chemical or electrical), e.g. for stroke, epilepsy, Parkinson's disease, and autism. Whether genetic or induced, these models are characterized by a complex reorganization of neuronal networks. These reorganizations are hypothesized to underlie the symptoms of the disorders and associated co-morbidities.

The Virtual Mouse Brain (TVMB) is an extension of TVB, designed to virtualize the brain of rodents and runs within the TVB framework. It includes the same generative models of brain activity and analysis tools. As for TVB, TVMB is open access. TVMB can be used to interpret neuroimaging data and assess causality (Fig. 2). When neuroimaging data is acquired in patients, clinicians get an ''instantaneous'' picture. Differences can be noted with a ''control'' population, but what do they mean? Since neurological disorders evolve in time, such differences may have occurred in a distant past, and may be unrelated to the pathological state. In addition, results from The Human Connectome Project strongly suggest that both connectome and whole brain dynamics, despite the existence of a general template, are unique to each healthy individual and characterize/predict their brain activity/performance [13–16]. Hence, differences may not be linked to a pathological state. The only way to address this central issue is to follow an individual from the pre-symptomatic to the symptomatic phase. At present, this is not realistic in the human population. However, such studies can be performed in rodent models.

In the case of induced models, we can obtain neuroimaging data before the brain insult that will lead to the pathology (Fig. 2). We have thus access to the ''control'' state of each individual, for example the brain connectome and resting state fMRI. More neuroimaging data can be obtained from the same animals at different time points, and alterations can be correlated with the appearance of phenotypic traits.

At each time point, each brain can be virtualized in TVMB, using DTI-based connectome data. The multiple generative models of the platform can be used to simulate electrophysiological activity or BOLD signal. Simulated data can then be used to interpret experimental data. For example, can changes in the connectome explain alterations in whole brain dynamics? Since we have access to the ''control'' state, it is possible to modify the ''control'' connectome in TVMB to match the altered connectome. This should change resting state dynamics, and if these changes match experimentally measured ones during the pathological period, it is possible to propose that alterations in the connectome are sufficient to explain changes in whole brain dynamics. It is even possible to explore which connections play a key role to explain the phenotype.

TVMB can then be used to generate testable predictions. It is straightforward to lesion some regions, cut some connections or stimulate axonal tracts *in silico*. A parameter search can be used to obtain a desired effect, for example on whole brain dynamics, at the level of each individual brain. Such simulations are not too time-consuming and predictions can be directly tested *in vivo* in the same mouse. Many tools are available to ablate regions/axonal tracts, activate and

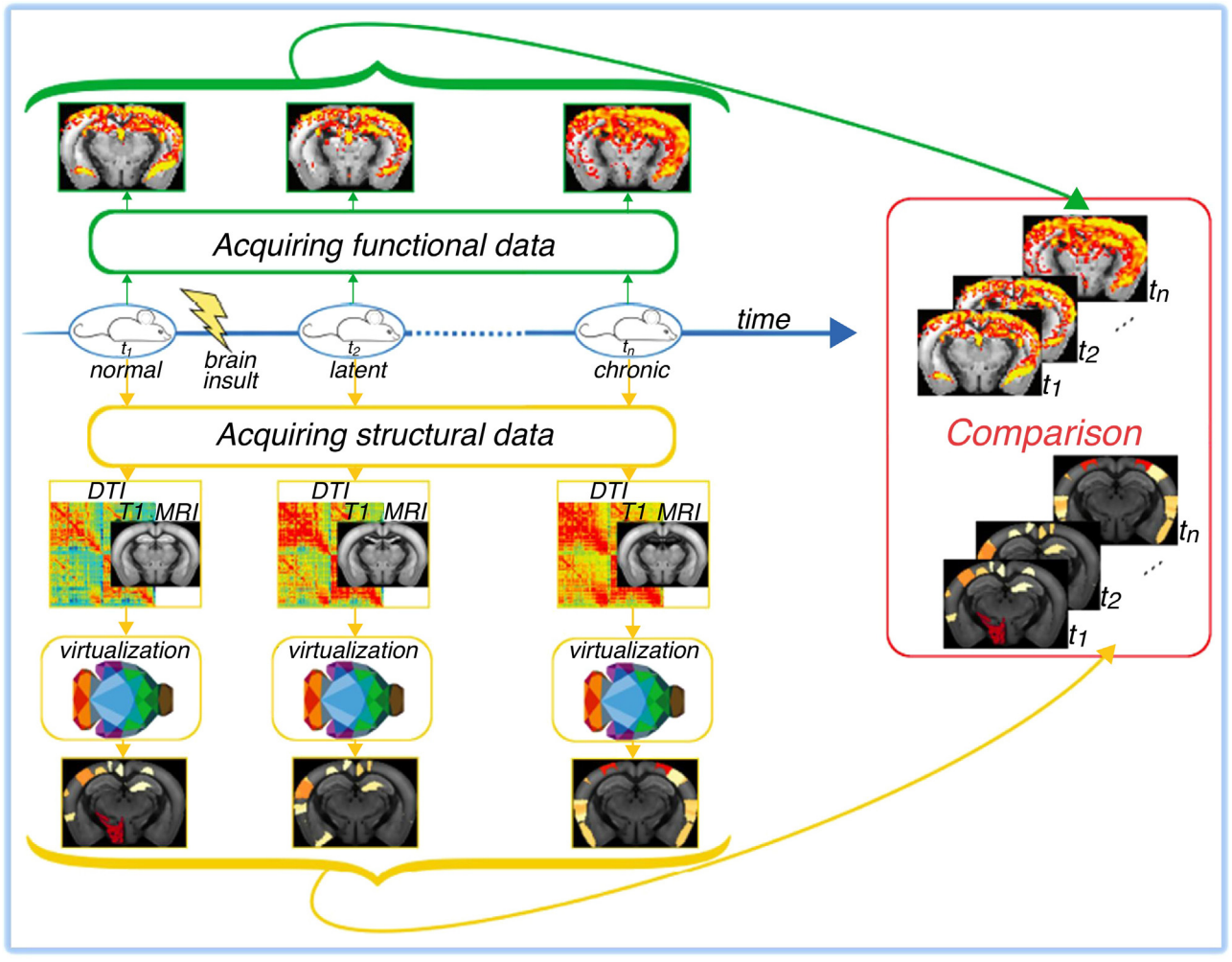

Figure 2. The Virtual Mouse Brain. Each animal is its own control and is scanned at different time points (shown here for experimental temporal lobe epilepsy) to obtain structural and functional data. The brain is then virtualized in TVMB to simulate resting state fMRI (as shown here), EEG, stimulation, seizure genesis and surgical procedures. The simulation results are compared to empirical data.

inactivate them, including optogenetics, which allow a fine control of neuronal networks. In the case of optogenetics, network manipulations are reversible, and different predictions (e.g. different patterns of activation/inactivation) can be tested experimentally.

Let us consider the case of epilepsy. We recently demonstrated a phenomenological model of seizures with partial onset, the Epileptor, which can be used to study seizure genesis and propagation [21,22]. The Epileptor comprises a set of differential equations of five state variables. One variable evolves slowly in time and can drive the network to seizure onset via a bifurcation, drive the course of the seizure and brings the system to seizure offset via another bifurcation. The Epileptor model accounts for a majority of seizures recorded in patients, and non-human species [17]. Once virtualized following the same strategies as in TVB [23,24] and respecting time delays via signal transmission, we can include Epileptors at each brain node to study seizure genesis and propagation. Then, a parameter search can be conducted to predict the best protocol to prevent seizure propagation, via stimulation or ablation of regions or axonal pathways, and test the predictions experimentally.

So far, we have considered the virtualization of individual mouse brains. The activity (electrophysiological or BOLD) generated in TVBM relies on the connectome obtained from DTI data. However, three major limitations of DTI exist, that is (1) our ignorance about the directionality of the connections, (2) the indirect nature of the measures of connectivity, and (3) the fact that only major axonal pathways can be identified [19,20]. How such limitations affect the simulations is not yet established. This is the reason why TVMB also includes, as a reference, a virtual mouse brain based on the very detailed mesoscale connectome from the Allen Institute for Brain Science [25]. This connectome was obtained via

detailed tracer studies. The main drawback comes from the averaging from multiple mice measured at one age, which removes the possible importance of the specificity of individual connectomes. However, the Allen Virtual Mouse Brain offers the possibility to explore the role of some connections that cannot be detected by DTI on whole brain dynamics.

Conclusion

Large-scale brain network modeling enables linking personalized brain models with patient-specific neuroimaging data [26,27]. Despite the enormous neuroinformatics complexity of integrating brain data, high performance computing and mathematical modeling, modern computational neuroscience provides in silico platforms (TVB, TVMB) for the testing of hypotheses of brain function on the large-scale system level. Suitable paradigms allow exploring questions linked to the network and the spatiotemporal dynamics it supports. Other questions are very limited, as many biophysical details are absorbed in collective variables. Nevertheless, these approaches present the first step towards personalized brain modeling and their power is yet to be explored.

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Computational Models of Neurological Disorder

Computational Drug Networks: a computational approach to elucidate drug mode of action and to facilitate drug repositioning for neurodegenerative diseases

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While computational approaches based on chemical structures have been extensively used in drug discovery, drug induced transcriptional responses provide a complementary view of their effects. Network visualizations facilitate the exploration of the chemical space in a comprehensive, integrated view. Systematic approaches can be particularly useful for repositioning drugs acting on the CNS, where polypharmacology, targets promiscuity and pharmacokinetic properties must be finely tuned. Here we present a review of the most recently developed methodologies for comparative structure-based and transcriptomics analyses together with applications to the field of Drug Repositioning. We also show an application example in which we searched for drugs and perturbagens inducing cellular autophagy, a suitable strategy to improve phenotype of neurological diseases.

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Introduction

Drug repositioning represents a convenient alternative to the classical drug discovery pipeline by identifying new therapeutic applications for existing marketed drugs [1]. During past decades, the main strategy for drug development has been high-throughput screening to identify compounds showing activity against single therapeutic targets or pathways. However, the ratio of successfully identified drugs to screened molecules has decreased dramatically [2]. The ''one drug, one disease, one target'' paradigm has been overcome by the more comprehensive polypharmacology and Systems Biology approaches, especially for CNS diseases. Moreover, integrated approaches can rely on multiple layers of biological information extracted from genes, pathways, targets and

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drugs as interaction networks [3,4], providing new alternatives for drug discovery and repositioning for neurological and CNS disorders from a systemic perspective.

Chemical networks

The classical computational medicinal chemistry approach investigates Structure Activity Relationships (SAR) between drugs, by structurally comparing them and searching for New Chemical Entities (NCEs) or repositioning. The concept is based on the chemical similarity principle milestone assessing that structurally similar drugs are likely to have similar biological activity and MoA [5]. Usually, in computational medicinal chemistry, a drug is represented as a set of physicochemical or molecular features (e.g., molecular substructures, chemical groups, atomic pathways that are responsible for biological activity) encoded by molecular fingerprints.

Drug chemical structure similarities can provide interesting opportunities for repositioning and target identification. The increasing amount of publicly databases of chemical structures and high-throughput screening data ([6–9]; see Table 1 of [10]) will be crucial sources of information for drug development methodologies in the next future. For instance, the PubChem database (ref.), one of the most famous source of drug biological and chemical information, contains over 100,000,000 compound and substance records linked to biological property information and bioassays for target identification collected from several scientific studies and analysis wolrdwide.

In order to manage the huge amount of data coming from the different annotation sources, network visualizations have been exploited in which nodes represent drugs and edges between nodes highlight relations such as significant similarity of the corresponding chemical structures or transcriptional effects. Network-based drug discovery aim to systematically investigate the space of small molecules in order to disclose their modes of action and/or identify innovative therapeutic treatments [2,11].

Chemical similarity networks (representing structurally similar small molecules as connected nodes in a network) have been applied to identify off-targets and metabolic effects and thus predict polypharmacology, side effects and novel therapeutic effects for several different compound sources and annotation databases [12–21].

One of the most recent uses of chemical similarity networks for drug repositioning regards CSNAP3D [22], a 3D upgrade of the CSNAP framework for large-scale network-based drug target prediction based on ligand superposition. A CSNAP3D analysis incorporating 2D and 3D similarity metrics led to the identification of peculiar pharmacophore features of HIVRT inhibitors Efavirenz, Nevirapine and Tivirapine, which are rather different in their molecular shape. The algorithm has been experimentally validated by analysing novel antimitotic compounds and identifying several low molecular weight microtubule-stabilizing agents that mimic the Taxol binding mode and exhibit anticancer activity.

However, drug-target interaction networks cannot be used to predict potential targets for new chemical entities, such as newly synthesized chemical structures or drugs failed in clinical trials. To this end, Wu *et al*. [23] proposed an integrated network and chemoinformatics tool, named Substructure Drug Target Network Based Inference (SDTNBI), for large-scale DTI (Drug-Target Identification) prediction and drug repositioning. SDTNBI uses chemical substructures, which are a set of features that can be shared by chemical structures, to bridge the gap between known drugs and new chemical entities. They were able to identify nonsteroidal anti-inflammatory drugs (NSAIDs) as novel anticancer drugs, targeting AKR1C3, CA9, CA12 or CDK2.

An important aspect in medicinal chemistry for brain disease regards drug optimization for brain penetrance (Brain–Blood–Barrier permeability). Drug bio-availability is a critical step in the development of CNS drugs for neurological and neurodegenerative disorders such as Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and encephalitis [4,24]. In this particular context, drug repositioning represents a particularly convenient strategy because compound profiles have already been pharmacokinetically optimized and approved in Phase I clinical trial [25].

Table 1. Top-10 drug neighbours of Sirolimus.

Rank	Drug/perturbagens neighbours	Mantra distance	Target	MoA
1	Wortmannin	0.547	PI3K	Anticancer
2	PI3K_Ex20dmso/inh_F	0.588	PI3K	
3	Quinostatin	0.614	PI3K	Anticancer
4	ERK CI1040 Panc2.13	0.659	ERK	Anticancer
5	Trifluoperazine	0.662	DRD2	Antipsychotic
6	Insulin reverse signature	0.67	IGF-1	
7	Latamoxef	0.678	pbpC	Antibiotic
8	Methylergometrine	0.689	DRD1	Antipsychotic
9	Emetine	0.716	40S ribosomal sub-unit	Antihelmintic
10	Co-dergocrine mesilate	0.717	ADRA2A-1A, 5HTRs, DRD1-2	AD/dementia

Despite their heterogeneous clinical phenotypes, CNS diseases may impact common molecular mechanisms such as oxidative stress, mitochondrial functionality, ubiquitin-proteasomal and protein metabolism pathways, all of which are known to associate with AD, PD and HD [4]. Sawada *et al.* [26] developed statistical models for predicting new drug indications by exploring the target space of drugs (including primary targets and off-targets) based on chemical structure similarity and molecular effects similarity. In particular, their model based on chemical similarity predicted Pioglitazone, a drug used to treat type II diabetes primarily targeting PPARPY (Peroxisome Proliferator-Activated Receptor gamma), to be effective for Parkinson disease for its off-target effect on MAOB (monoamine oxidase type B). Mechanistically, this is reasonable since inhibition of MAOB increases dopamine levels in the brain and some anti-Parkinson drugs are MOAB inhibitors.

A very recent application based on network pharmacology together with docking-based virtual screening was used by Ke *et al.* [27] for classifying natural products known to be effective in neurodegenerative diseases. The large dataset was composed by 58,147 compounds collected from the Universal Natural Products Database (UNPD), the Chinese Natural Products Database (CNPD) and Reaxys. Docking-based virtual screening helped in predicting the efficacy of the substances on neurodegenerative disease targets extracted from the Therapeutic Target Database (TTD) and DrugBank related to neurodegenerative diseases (dementia, Alzheimer, Parkinson and Huntington disease).

Transcriptional networks

Although drug intrinsic features, such as chemical structure or physico-chemical properties, are powerful descriptors to define and search a chemical space, they are not necessarily able to capture drug-induced molecular effects. Drugs with similar structure may exert different effects due to off-targets, while drugs with different structure can induce similar effects by affecting the activity of different genes in the same pathway. For these reasons, approaches directly based on induced effects at the transcriptional level have been developed. In particular, the ever-increasing availability of transcriptomic data has made possible to compare drug effects at the molecular level in a systematic way. On the other hand, transcriptomic data suffers heavily from technical and biological variability, making the assessment of a drug-induced cellular state difficult. This challenging problem has been mainly tackled in two different ways: performing normalization on heterogeneous datasets or building large homogeneous datasets of expression profiles.

Gene expression normalization, and batch effect correction in particular, is a well-studied topic. One interesting pointer in literature regards the limma (Linear Models for Microarray Data [28]) package, classically used to assess gene expression significance and recently explored for broader normalization applications, including batch effect correction. Large repositories of heterogeneous gene expression data such as Gene Expression Omnibus (GEO) or ArrayExpress [29] support forms of data format standards such as the MIAME, but are not designed to be used as bulk collections of profiles. Nonetheless, tools trying to abstract study-specific data and provide a single interface to all the data have been developed, the most recent of which being SEEK (search-based exploration of expression compendia, [30]). An alternative approach is provided by CREEDS (CRowd Extracted Expression of Differential Signatures, [31]), for which crowd-based manual curation was adopted.

Concerning homogeneous-by-design collections of gene expression datasets, the most popular is probably the Cmap (Connectivity Map, [32]), including gene expression profiles for 5 different cell lines treated with 1309 different small molecules at varying concentrations for a total of 7056 Microarray experiments. All Cmap data were produced following the same partly automated protocol, thus minimizing experimental bias. Many current tools, including some that will be mentioned in the following, are based on these data. A new version of the Cmap (LINCS, Library of Integrated Cellular Signatures) including gene expression profiles for ~5000 small-molecule-compounds and ~3000 genetic reagents for a total of ~1.5 M profiles is currently being released. A partial release including 115209 profiles has been published on the GEO website (GEO accession: GSE70138). LINCS data have recently been used to build an adverse drug reactions classifier based on integrated gene expression and chemical structure features [33].

One of the most popular transcriptomics-based drug network tools is MANTRA (Mode of Action by Network Analysis) [34]. Based on Cmap data, MANTRA is an online tool supporting the navigation of a drug network in which links are defined by transcriptional response similarities. MANTRA attempts a cell-line neutral approach, allowing to exploit more publicly available data at the cost of diluting cell type specific mechanisms. The website also allows the creation of new nodes built from gene expression profiles submitted by the users and made available to the public. Mantra has been used also recently for a number of different applications: to reposition Niclosamide and Pyrvinium Pamoate, two anthelmintic drugs, as inhibitors of oncogenic PI3K-dependent signalling [35]; to screen for 16 quinolone-like molecules with the aim of overcoming drug-resistance in Salmonella typhimurium [36]; to identify Mometasone as a corrector of DF508-CFTR mutated protein causing cystic fibrosis (CF) in CFBEo-cells [37]. Note that for the CF application, a non-chemical seed was used (an expression profile derived from low temperature treatment), which well exemplifies how systemic approaches can be applied without any assumption about a specific seed compound or known mode of action.

This approach can be particularly interesting for complex neurological disorders.

A major drawback of drug networks is the fact that, while they are able to unravel possibly unexpected similarities between drugs or between drugs and other types of perturbagens, they do not address the problem of providing a biological explanation for such predicted links. The Drug-set Enrichment Analysis (DSEA [38]) is a tool developed to tackle this specific problem. DSEA aims at highlighting common pathways among those that are dysregulated by a set of drugs, exploiting Cmap data as a statistical background. Thus, given a set of drugs, the tool searches for pathways that are significantly dysregulated by them. In this way, DSEA is able to dilute strong transcriptional signals of each drug that are not relevant for the common therapeutic effects of the drug set. For example, the tool was used to identify the upregulation of chloride channel related genes as a common mode of action across a set of 10 different drugs known to be partly active in rescuing CFTR mutated protein, a chloride channel causing cystic fibrosis when defective [38]. DSEA has also been used to characterize a new computationally identified set of drugs repositioned as antiepileptics and to demonstrate how they, although pharmacologically heterogeneous, may constitute a class when considering the modulated pathways [39].

Enhancing autophagy as potential treatment for neurodegenerative disorders

The accumulation of misfolded proteins in neurons is a prominent feature of neurodegenerative diseases [40,41]. Autophagy, the catabolic process that facilitates nutrient recycling via degradation of damaged organelles and proteins through lysosomal mediated degradation [42], is particularly relevant in maintaining neuronal health. The reason is that several potentially harmful proteins (e.g., α-synuclein and huntingtin) are autophagy substrates and accumulate in neurons when autophagy is impaired [43]. Furthermore, Komatsu *et al*. demonstrated the involvement of autophagy in neurodegenerative diseases by knocking out Atg7 autophagy-related mice gene [44], showing symptoms of neurodegeneration in the central nervous system, while mutation in the human ATG5 gene caused development delay and ataxia [45]. For a more complete overview about defects in autophagy causing neurodegeneration see Refs [40–43,46].

Lysosomal dysfunction has been associated with many neurodegenerative diseases, including common, late-onset forms of neurodegeneration (Parkinson's disease – PD, Alzheimer disease – AD, and Huntinghton disease – HD, see Ref. [43]). The 'amyloid hypothesis' has driven the search for drugs that stop aggregation of pathogenic beta-amyloid, which generates potentially toxic oligomers and plaques, but so far these efforts have not led to a successful disease-modifying treatment [47]. Huntinghton disease HD is an inherited brain condition caused by mutations in protein huntingtin (HTT) that lead to abnormal and toxic protein forms due to polyglutamine expansions. Expanded HTT may affect the efficiency of autophagy [43]. Pharmacological induction of autophagy in HD mouse and fly models ameliorated the phenotype of the disease [48,49]. α-Synuclein accumulation and aggregation play a central role in the pathophysiology of Parkinson's disease PD and in a subset of neurodegenerative conditions known as synucleinopathies. The aggregated forms of α-synuclein binding lysosome disrupt its activity [43].

Therefore, enhancing autophagy and thus neuronal clearance may represent a potential treatment for neurodegenerative disorders. One of the possible strategies to modulate autophagy is through the mTOR complex [42,50]. The inhibition of mTORC1 protein by Sirolimus (also known as Rapamycin) stimulates autophagy.

We now outline a practical example of computational drug repositioning to illustrate the application of a genetic signature network-based method for neurodegenerative disorders by enhancing autophagy. Following the many-to-many paradigm, as opposed to single-drug single-target approach, this example does not assume any specific mode of action of the seed compound, but rather explores the drug space to search for other molecules possibly inducing similar effects at the transcriptomic level. Insights about such effects are investigated a posteriori. In particular, we start using Sirolimus as seed compound in the Mantra tool [34] and then apply the DSEA tool [38] to understand phenotype-specific biological pathways shared by the set of Sirolimus drug neighbours, helping to formulate hypotheses on the MoA. Sirolimus gene signature associated with activation of autophagy pathway may offer novel opportunities for targeted therapeutics discovery for neurological disorders caused by the accumulation of harmful proteins and protein aggregates since accumulate in neurons when autophagy is impaired.

48 drugs and 12 perturbagens (Fig. 1) were found significantly similar to Sirolimus (Mantra distance < 0.8). Those drugs constitute the set of candidates for repositioning as autophagy enhancers through mTOR pathway inhibition. Among the top-10 neighbours, four inhibit the PI3K pathway which is an importan upstream major target of the mTORC1 complex (Table 1). The PI3K/AKT/mTOR/-dependent pathway regulates cellular growth, autophagy and apoptosis involved in synaptic plasticity and trasmission [51].

Interestingly, the insulin-reverse gene signature (GEO accession: GSE26834) was found at 6th position among Sirolimus neighbours. It has been reported that inhibition of the insulin/insulin growth factors (IGF) signalling pathway increase lifespan and protects against neurodegeneration in model organisms by acting upstream the mTORC1 complex [52,53]. Thus, the reverse gene signature of insulin mimics the inhibition of the IGF-1 pathway that induces autophagy, providing an empirical validation of the approach.

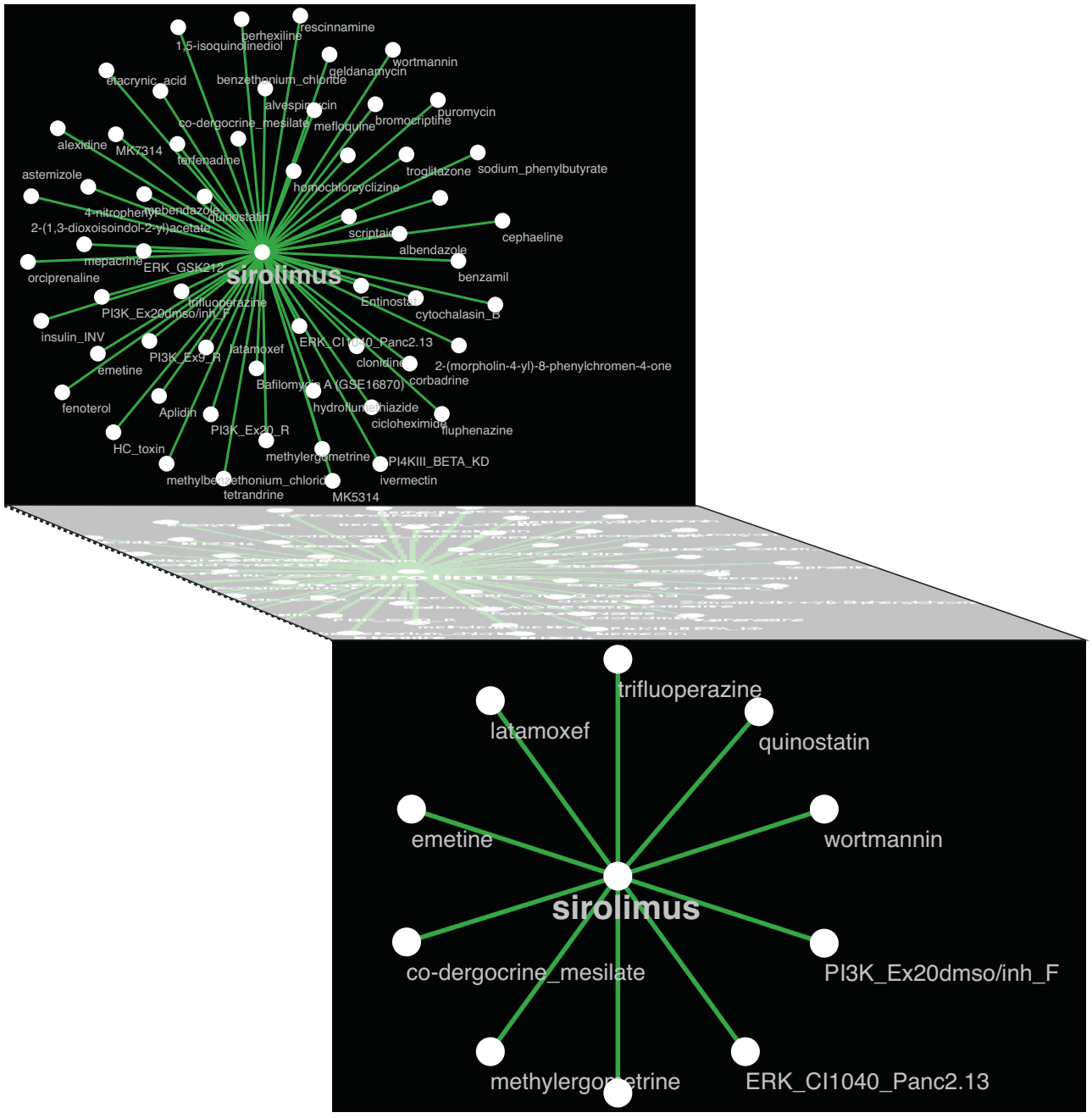

Figure 1. First phase of the analysis example. Transcriptional neighbours of the mTORC1 inhibor Sirolimus (Rapamycin) are selected as repositioning candidates. (top) 48 drugs and 12 perturbagens were found at a significantly small distance (0.8). (bottom) Focus on the top-10 neighbours.

The ERK pathway modulated by CI1040 inhibitor (GEO accession: GSE45757) is reported to be another important autophagy modulator of the mTOR pathway [54,55].

Moreover, co-dergocrine mesilate, an ergoloid derivative drug ranked 10th, has already been applied for AD and dementia, although through an unknown mechanism [56,57] (Fig. 2).

Next we applied the DSEA tool [38] to detect molecular pathways that are consistently up- or down- regulated by the set of 6 drugs reported in Table 1. Among the top-10 position in the Gene Ontology – Cellular Component database, we found the HOPS complex (3rd position), the autophagic vacuole (4th position) and the late endosome membrane (5th position) pathways that are significantly up-regulated (p-value < 0.01) and related to the autophagic process. This result confirms the involvement of autophagy pathways as common effects of the Sirolimus transcriptional neighbours, further suggesting a possible repositioning for those drugs as autophagy enhancers for neurological disorders (Table 2).

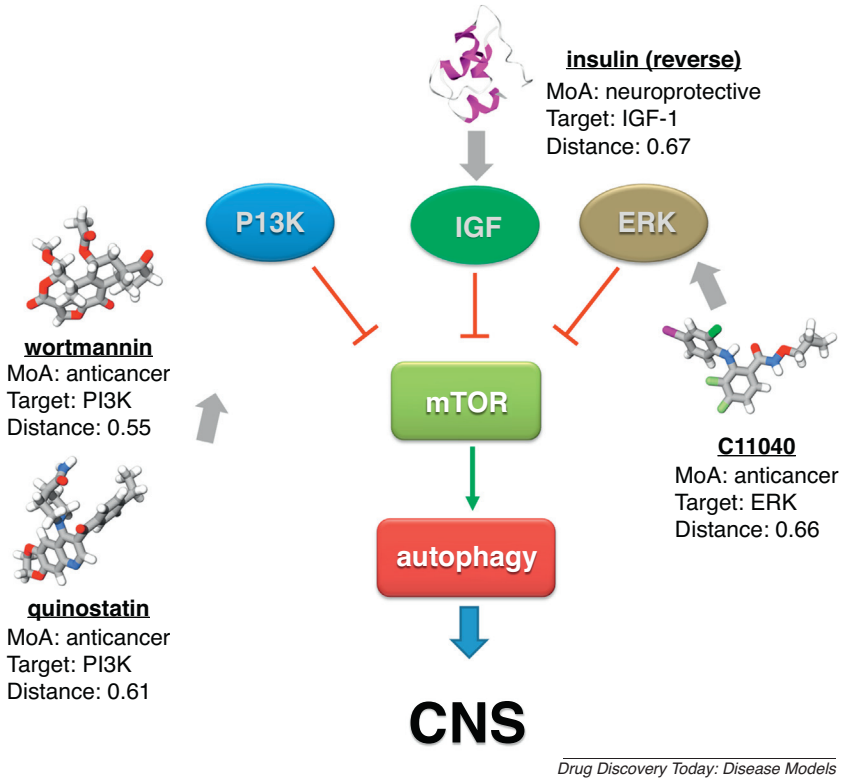

Figure 2. Summary of the example analysis results. Molecular structures and targets of the drug/perturbagen neighbours of Sirolimus promoting autophagic processes.

Table 2. DSEA of the 6 drug neighbours reported in Table 1.

Rank	CC pathway name	ES	*p*-value
1	Mitochondrial nucleoid	−0.81	9.71e-5
2	Nucleolus	−0.79	2.12e-4
3	HOPS complex	0.76	4.09e-4
4	Autophagic vacuole	0.76	4.35e-4
5	Late endosome membrane	0.75	5.54e-4
6	Exocytic vesicle	−0.74	7.46e-4
7	Nuclear pore	−0.73	9.05e-4
8	DNA replication factor C complex	−0.71	1.34e-3
9	bleb	−0.71	1.55e-3
10	COP9 signalosome	−0.71	1.64e-3

Conclusion

In this review we have discussed how computational drug networks can aid and facilitate drug repositioning. Chemical- and transcriptional-based drug networks are able to effectively summarize complex information concerning intrinsic and extrinsic drug properties. A special focus on the main neurodegenerative diseases has been presented and, in particular, an example of drug repositioning using a combination of the transcriptomics-based tools MANTRA and DSEA demonstrated the potential of such approaches in identifying drugs sharing similar MoA. The ever-increasing availability of −omics data will likely make possible to perform more efficient and system-specific predictions in the near future.

Conflict of interests

We have no conflict of interest to declare.

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Computational Models of Neurological Disorder

Computational modeling to improve treatments for essential tremor

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Essential tremor (ET) is a neurological disorder of unknown etiology that is typically characterized by an involuntary periodic movement of the upper limbs. No longer considered monosymptomatic, ET patients often have additional motor and even cognitive impairments. Although there are several pharmacological treatments, no drugs have been developed specifically for ET [1], and 30–70% of patients are medication-refractory [2]. A subset of medication-refractory patients may benefit from electrical deep brain stimulation (DBS) of the ventral intermediate nucleus of the thalamus (VIM), which receives cerebellar inputs. Abnormal cerebellar input to VIM is presumed to be a major contributor to tremor symptoms, which is alleviated by DBS. Computational modeling of the effects of DBS in VIM has been a powerful tool to design DBS protocols to reduce tremor activity. However, far less is known about how these therapies affect non-tremor symptoms, and more experimental and computational modeling work is required to address these growing considerations. Models capable of addressing multiple facets of ET will lead to novel, more efficient treatment.

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Introduction

ET is the most common neurological movement disorder that affects 4–5% of the adult population [3,4] and is up to twenty times more prevalent than Parkinson's Disease (PD) [5]. Upper limb tremor, and occasionally head and neck tremor, are the predominant symptoms of ET. Limb tremor usually appears with movement, sometimes with sustained postures, and is not usually present at rest [6,7].

Despite mounting evidence to the contrary, ET is still often described as 'benign', an antiquated term that referred to the idea that ET was thought of as monosymptomatic and non-debilitating [8,6,9,10]. In reality, ET is a progressive disorder, possibly neurodegenerative, and can present with additional motor deficits of tandem gait and balance for up to half of patients, consistent with evidence implicating cerebellar pathology [11–13] (and see [9,14]). The precise mechanism by which these deficits contribute to tremor and non-tremor symptoms are not fully known [6,15].

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Postural and intentional tremor are clearly the most prominent symptoms, but other non-tremor motor deficits have also been noted [16–22], though they are less obvious than the primary symptom of tremor and sometimes subclinical [6]. The most common problems concern tandem gait abnormalities [20,22,23], but several additional deficits have also been observed, including difficulty maintaining postural control [16], abnormal timing of ballistic movements [17], impaired finger tapping [18], impaired eyeblink conditioning [19], and deficits in motor learning [21].

Additionally, a growing body of work has demonstrated non-motor, cognitive symptoms in ET patients [24,25], including depression [26], apathy and anxiety [27], and changes in personality [28]. Tremor can even be detrimental for patients to the point of substantial social anxiety and depression [6,29]. Some studies have also suggested an increased probability of ET patients being diagnosed with Parkinson's Disease (PD) (reviewed in [30]). Regardless of whether ET is a neurodegenerative disorder [31], it is more complex than traditionally believed [6,32], and this growing body of evidence represents an opportunity for additional experimental and computational work to understand and define the disease more fully, toward improving all aspects of treatments.

Deep brain stimulation can be an effective treatment for tremor

The first line medications to treat ET include primidone, a barbituate whose metabolite acts as a $GABA_A$-receptor agonist, and propranolol, a beta-adrenergic agonist with possibly both central and peripheral action [1]. Several additional drugs such as the anticonvulsant topiramate have been tested, but to date, no drugs exist that are specific to treat ET [1,33]. For those patients that do not respond to medication [2], deep brain stimulation (DBS) is sometimes available as a treatment to alleviate tremor.

Electrical stimulation of the thalamus for cessation of tremor was demonstrated in the 1960s [34], and the modern era of DBS in ET and PD was heralded in the 1980s [35,36]. DBS is also being investigated to treat Alzheimer's Disease [37,38], depression, obsessive-compulsive disorder, and other neurological diseases (see [39]). In its present form, DBS involves stereotactically guided surgical implantation of a multi-contact electrode at the stimulation site, connected to a subcutaneous stimulator and battery implanted in the chest wall just under the clavicle.

In most cases, DBS of the ventral intermediate nucleus of the thalamus (VIM) is an effective treatment of tremor in ET, but some patients do not respond [40], and DBS is sometimes associated with certain deficits in gait and balance [41] or speech [42,43] and can become less effective over time [44,45]. Stimulator programming is typically performed by a neurologist to minimize tremor while avoiding side effects such as paresthesias, but it is not clear to what extent additional symptoms are also treated.

The evidence on the efficacy of DBS to treat gait and balance issues is also not clear. With both unilateral and bilateral DBS, increased problems with gait have been reported [46], but these differences may be highly individual [41]. Optimizing stimulation to address this problem along with tremor may be helpful, as one report found that overstimulation led to increased problems with gait that was not present at reduced stimulation levels [23,47].

Ultimately, DBS provides tremor relief in particular for a substantial portion of patients, but its impact on other symptoms is less well understood. DBS may be improved with a better mechanistic understanding of tremor and non-tremor symptoms of ET, and computational modeling has been a valuable tool in designing operational DBS parameters. To date, DBS modeling studies have focused largely on reducing tremor rather than non-tremor symptoms. Here we review computational modeling efforts that have helped to shape understanding of DBS mechanisms. We propose that future improvements in DBS for movement disorders can leverage prior modeling insights and benefit from experimental and computational studies considering non-tremor symptoms.

Overview of computational modeling

Computational modeling relies on mathematical description of activity in the brain to identify underlying mechanisms and to provide testable hypotheses for experiments (Fig. 1). In particular, conductance-based network models strive for a description of cellular and circuit-level interactions that underlie brain activity, such as oscillatory electrical signals or other dynamically changing states (Fig. 1a and b). These models often rely on a system of ordinary differential equations describing the membrane dynamics of individual cells and their synaptic interactions [48,49]. Of course, models necessarily represent reduced representations of activity; as such, they are often difficult to constrain meaningfully [50], and no perfect model exists. Nevertheless, just as animal disease surrogates are invaluable to biomedical research, it is possible to utilize computational modeling with specific constraints derived from human and non-human primate data where available to accurately capture relevant features of neural activity. This approach may accelerate the development of novel therapeutic targets and strategies and may be more effective than experimental work alone.

Leveraging computational models for understanding ET

A growing number of computational models have been published to address various aspects of dysfunction and treatment in ET, with most focused on understanding how pathological tremor oscillations in the brain are suppressed by DBS. Yet to our knowledge, none of these models have

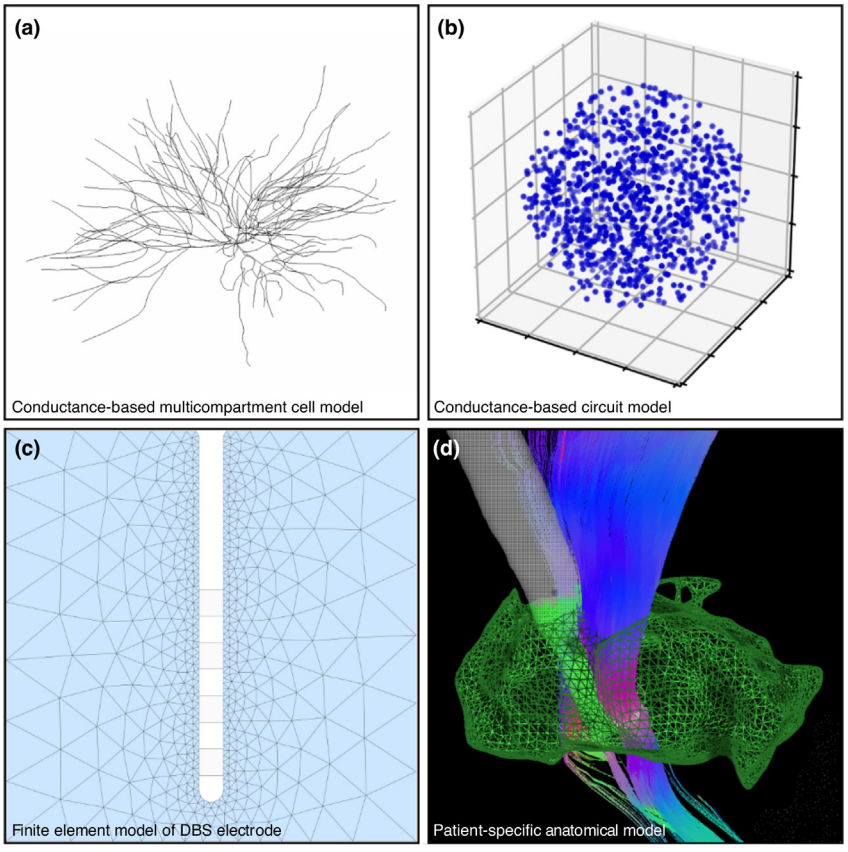

Figure 1. Multiple scales of computational models. Integration across model scales can provide novel insights into mechanisms of DBS and improve therapeutic strategies. **(a)**. Two-hundred compartment single thalamocortical relay cell with Hodgkin-Huxley-type dynamics [81]. **(b)** Circuit modeling one-hundred uniformly distributed random cell bodies simulated as single compartment Hodgkin-Huxley-type neurons. C. **(c)** 2D FEM mesh around a Medtronic Model 3387 electrode of electric fields, estimated with PyDistMesh 1.2 [82,83]. **(d)** Patient-specific anatomy with DBS electrode from intraoperative CT (gray), thalamic nucleus (green), and diffusion-based tractography (multicolor). Images registered using Waypoint Navigator and AFNI [84] and visualized in SUMA [85].

aimed to address non-tremor symptoms of ET that appear to be highly prevalent and not necessarily related to a specific oscillatory mode. Numerical simulations of the effects of DBS have been successful in helping to understand what was historically described as 'benign essential tremor'; our present understanding of the far more complicated profile of ET beyond tremor represents an opportunity for computational modeling to work toward understanding how non-tremor symptoms are affected by DBS.

Multiple levels of mathematical description exist to characterize the electrical fields induced by DBS and the resultant impact on neural circuits. The most descriptive of the field effects of DBS takes into account specific electrodes [51,52], including spatial geometry and materials of the electrodes using finite element modeling (FEM) in 3-space [53] and has shown the importance of explicitly accounting for tissue and electrode capacitance [54] and impedance [55] (Fig. 1c). These detailed models typically calculate the electric field due to the DBS electrode stimulation and apply it to a separate neural model, though these steps can also be done in the same framework, with each approach valid under specific assumptions [56]. Often, model simplifications are possible and informative; under certain assumptions, the electric field from the DBS electrode reduced to a point electrical source [45]. Further model simplifications have also been considered, which can have the advantage of being amenable to mathematical analysis. In the simplified models, DBS is often represented as an injected current, an additive term to the membrane voltage equation for cells [57,58].

Cells stimulated by DBS are often modeled with a class of compartmental models representing different cell structures (e.g. soma, dendrites, axons) using Hodgkin-Huxley-type systems of coupled ordinary differential equations [59] (Fig. 1a and b). The FEM electrical field solution of the DBS model is applied as an extracellular current term to cell compartments. These models have been successful at a variety of spatial scales, from the level of understanding the impact on cellular components to a broader level description

of the effect of stimulation on the dentatorubrothalamic tract (see [60]).

In ET, tremor frequency activity in the VIM is often considered as a necessary waypoint toward expression in the affected limb. An attractive candidate mechanism for DBS-mediated tremor relief in ET (and PD) is that the high frequency stimulation disrupts transmission of a tremor frequency signal in the thalamus to downstream targets [61,62]. Indeed, reduction of pathological oscillatory activity may be a hallmark of successful DBS in other areas as well.

Models revealed that axons and cell bodies respond differently to stimulation

An important effort of DBS modeling related to ET has been to understand the biophysical mechanisms of DBS on cellular and subcellular components [53,63], which has not been confined to VIM but also other targets of DBS, such as the GPi for bradykinesia [64]. The FEM approach described above has helped to clarify early questions regarding whether the principal action of DBS was inhibitory or excitatory. DBS was postulated to be inhibitory because the outcome was similar to that of an irreversible tissue-ablating lesion [65,66]. However, downstream recordings observed an increase in activity during the stimulation, which led to the apparent contradiction. Applying FEM and cellular modeling, McIntyre and colleagues demonstrated that extracellular high frequency stimulation from DBS may inhibit the cell soma while simultaneously exciting axons [53]. Stimulation lower than the threshold for activation of cells in VIM was found to disrupt somatic activity but drive axonal responses.

Disrupting transmission of a tremor signal is consistent with the clinical evidence that thermal ablations in thalamus can also be effective at alleviating tremor [61,62]. It is not yet clear whether it is the reduction of tremor frequency activity in VIM, the regularization of output, the effect of antidromic drive, or perhaps several effects, that is important in reducing physiological tremor, and the ultimate source of pathological tremor oscillatory activity in VIM remains to be determined fully [67].

Models help to improve DBS stimulation parameters

Another well-established avenue of computational modeling for ET is aimed at optimization of DBS stimulation itself based on understanding its impact on the biophysical activity of cells and circuits within the VIM (Fig. 1a and b). Presently, DBS parameters (stimulation amplitude, pulse width/duty cycle, frequency, electrode contacts, polarity) are selected by a trained clinician but largely based on trial and error for a specific patient, reducing tremor symptoms in the clinic while minimizing obvious side effects, such as paresthesias, dysarthria, and ataxia. A more principled understanding of the effects of these parameters on their neural targets may lead to more automated programming, battery efficiency, and ultimately better outcomes, an important step toward fully closed-loop adaptive systems [53,68,69].

Grill and colleagues, through a series of studies, have performed experiments and various scales of simulations to understand the role of temporal regularity in the efficacy of DBS [63,70–72]. They found that temporally irregular patterns, while able to reduce tremor, did not perform as well as regular patterns and have found that masking cerebellar burst-driving inputs to thalamus may be crucial in reducing tremor. Further investigation revealed that the pause length was important in determining whether tremor was effectively suppressed, and their computational modeling suggested that the rebound bursting attributed to tremor-like pathology had emerged during pauses [72]. Interestingly, non-regular patterns of stimulation were found to improve finger tapping in PD patients and simultaneously reduce pathological oscillations [73], demonstrating the possibility of disease and location-specific effects that should be considered in designing stimulation therapies. Novel pulse shapes have also been simulated, resulting in principled predictions that non-rectangular pulses may provide more efficient tissue activation [74,75].

Model-informed control of tissue activation volumes may lead to improved, patient-specific therapy

Recent modeling efforts have identified patient-specific strategies that combine magnetic resonance imaging-guided tractography with realistic FEM models of DBS stimulation [76–78] (Fig. 1d). DBS electrodes that enable granular, directional control over current (i.e. current steering) have been employed to change the activation volume and increase the amplitude of stimulation while avoiding regions associated with side effects [79,80]. These tools enable directionally specific stimulation to reduce tremor (in the case of ET) while avoiding side effects that may arise from stimulation of nearby structures. This approach may also facilitate investigations into the origin of non-tremor symptoms and designing neuromodulation strategies to address them.

Future opportunities for computational modeling to improve ET treatment

Crucially, modeling DBS in ET would benefit from an improved understanding of the basic mechanisms giving rise to movements (including tremor) in the modulated circuit. Incorporating additional work on connectomics of brain areas implicated in ET pathology into network models may also help to drive development of new hypotheses [62]. Understanding the normal role of the cerebellar–thalamic–cortical pathway in motor behavior and motor learning will likely yield additional insight into the modes of its failure, giving rise to tremor as well as other, more subtle, motor deficits. Once these mechanisms are understood, rational

neuromodulation strategies can be designed to address these specific circuit perturbations.

To this end, while substantial efforts have gone toward modeling the effect of DBS on various neural targets and have helped to shape present DBS protocols, comparatively few models have been created to address specific facets of ET pathology outside the context of the effect of DBS on tremor. Experimental work to study non-tremor symptoms has been increasing in recent years, and computational modeling has an opportunity to do so as well.

We suggest that further efforts in computational modeling of all aspects of ET pathology can lead to novel testable predictions on improved methods to reduce tremor as well as non-tremor symptoms. These efforts should integrate across scales of modeling and focus on both the identification of novel pharmacological treatments and electrical stimulation therapies that will ultimately improve patient care.

Conflict of interest

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Computational Models of Neurological Disorder

Computer modeling of epilepsy: opportunities for drug discovery

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Analysis of the brain as a dynamical system can assist drug development for dynamical diseases such as epilepsy. The pathological trajectories that make up a seizure differ significantly from the physiological trajectories of normal brain function. These trajectories depend on parameters – conductances and time constants of ion channels and synapses – that can be modified by drugs. Drug development will benefit by taking account of the way in which multiple parameters – multiple drug targets – produce trajectory alterations. This may lead us to reconsider potential benefits of multi-target polypharmacy, of drug cocktails, and of so-called 'dirty drugs' (drugs with activity at multiple locations).

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Introduction

Multi-target pharmacological treatment through polypharmacy is used empirically for brain disease without good understanding of the interrelated effects of drug combinations. These limitations reflect the difficulties in understanding interactions in the brain, a complex nonstationary organ where both physiological and pathophysiological interactions span orders of magnitude both in space and in time. Multiscale computer modeling represents an effort to begin to master this complexity through simulation. Epilepsy is complex in both cause and manifestation, having multiple disease subtypes. Traditional evidence-based medicine (EBM) can only take us so far, since EBM works best when dealing with a clearly defined unitary disease where one or a few medications are being considered that act at only one or two sites. In epilepsy, the combinatorics of multiple drugs being used variously against multiple sites for multiple disease subtypes at many different stages of pathological developments provides a combinatorial explosion of situations to be studied.

The notion of a 'river of epilepsy' (Fig. 1) dates back to the work of Lennox and others from the mid-19th century. This concept was used to distinguish epilepsy from the prevailing notions and hopes that most diseases would follow the single-hit model seen with infections. In infectious disease, Koch's influential *postulates* focused on this one-hit notion of disease. If the causative infectious agent, typically a bacterial strain, was present, the patient would develop the disease. If that specific agent was not present, the disease could not develop. What Lennox noted was that epilepsy was not at all like that – patients developed the disease based on a confluence of factors. No one factor, no one agent, could be identified as being *the* critical causal factor. Ironically, the solution of the human genome only

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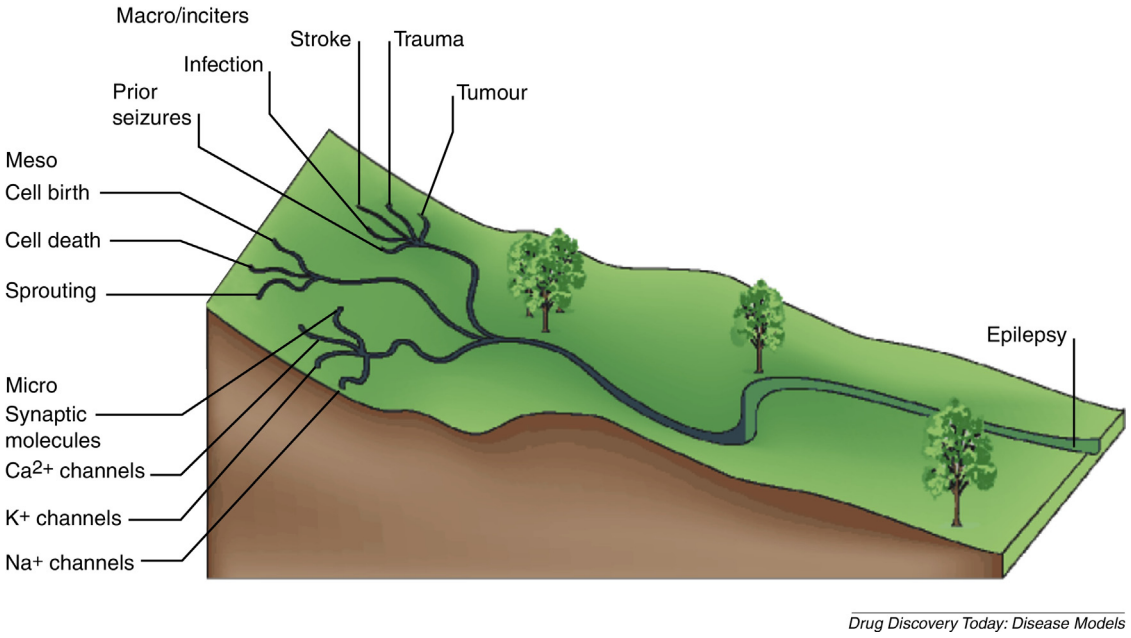

Figure 1. The river of epilepsy based on many contributing factors which will include a patient's genetics as well as experience. Historical factors of importance may include head trauma, drugs, response to prior injury, chronic or acute ischemia, etc. (With permissions from Lytton 2008 [1] Modified from Lennox and Lennox [2].)

reinforced Lennox's interpretation for epilepsy, as well as for other brain diseases. Only rarely can a single mutation be identified as the single major causal factor. Instead many complex diseases are polygenic, a result that was not anticipated when the human genome project was initially projected as having the potential to identify disease carriers who could then be subject to prophylactic treatment to prevent development of full pathological manifestations.

Epilepsy is polygenic, polycausal and polyscale and can best be studied using the multiscale, multifactorial techniques available by applying mechanistic multiscale modeling to data obtained from a variety of *in vivo* and *in vitro* epilepsy models, as well as from clinical material. Factors that should be taken into account in such models would include various types of brain plasticity, alterations in ion channel composition of cells based on both genetics and on varying phosphorylation states determined by neuromodulators, changes in synaptic connectivity, damage to subpopulations in response to brain trauma, and other factors [1]. The clinician, and the drug developer, must consider how this mix of factors produces disease and what combination of countervailing factors could prevent seizures. Given the many causes, treatment may in many cases require drug cocktails which would ideally be precisely worked out in a way to provide complementary interventions for prophylaxis, for prevention of exacerbation, as well as for prevention of seizures.

Epilepsy is a dynamical disorder. Dynamical study can connect the kinetics of activations of onset, offset and metabolism of drugs with the set of dynamical tools that can be used to simulate electrophysiology and chemophysiology in the brain. A dynamical system, originally referring to physical motion, now refers to the many systems that evolve in time – weather, climate, physiology, etc. For the brain, the *state variables* which change over time would be neural membrane voltages at various locations, ion concentrations and states and concentrations of signaling proteins. Significantly, this includes ion channels and synaptic receptors that are affected by anti-convulsant drugs. By analogy with the dynamics of planets, the change in state variables can be described as trajectories, which can then be identified as either physiological or pathological trajectories, which are associated with different *parameter* settings in the models. These parameter settings can be modified by application of drugs that server to set up a system that is consistent with physiological trajectories. However, note that many dynamical systems, including the brain, can show bistability. In a bistable system, two different trajectories are consistent with the same parameters so that a system can jump between physiological and pathological due to the application of noise to the system [9].

Dynamics can also be described in terms of interlocking systems of positive feedback and negative feedback loops. Multiple feedback loops in the brain produce outcomes that are non-intuitive. Some feedback loops end up being partially compensatory and reduce disability, but others may end up

worsening the pathology. Similarly some drugs that are useful in particular kinds of epilepsies can exacerbate other types. For example, benzodiazepines increase inhibition and are used as ancillary or acute treatment in several epilepsies. However, in absence epilepsy, this increased inhibition can facilitate firing through mechanisms involving the T calcium channel, a calcium channels that is deinactivated by hyperpolarization, thereby producing increased cell firing and exacerbating seizures [10–12].

The many causes and many manifestations that characterize epilepsy can be organized in terms of the spatial and temporal scales of organization of the brain (Fig. 2). Brain function is prone to disruption at these many scales and such disruptions and reactions to the disruptions will interact both within and across scales. Temporally, relevant scales range from the millisecond scale of neural spike signaling to the multi-year scale of brain development and, later, degeneration. Spatially, a fundamental scale is the molecular scale where neuropharmacological agents act. These agents can then make changes that are expressed across scales. Chemical signaling via second messengers will elaborate many of these pharmacological signals and spread their influence throughout the cell, while other pharmacological agents will act on membrane channels and thereby rapidly spread their influence through effects on electrophysiological properties. From there, effects will be propagated upward as alterations of cell firing influences local, areal, and brain-wide network properties; and thence propagated back downwards as these changes alter synaptic efficacy, network and cell firing patterns and cell chemical signaling through adaptive, plastic changes at all these levels.

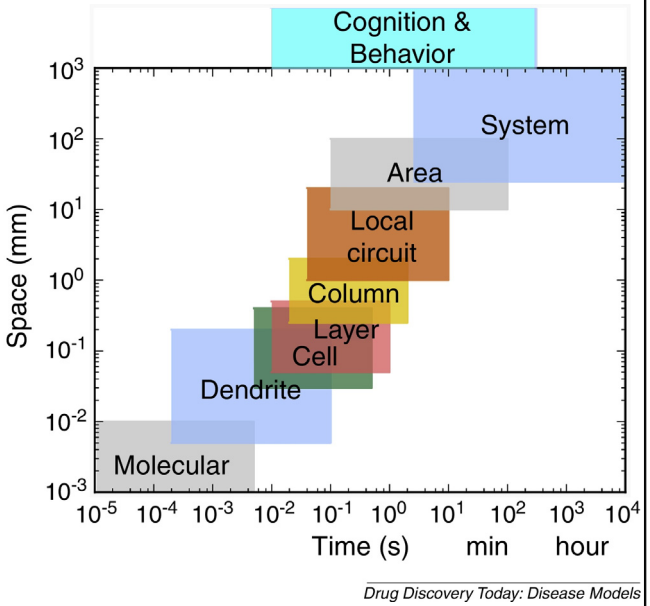

Figure 2. Treatment of epilepsy occurs at the molecular level of pharmacological intervention. Measures of the consequences of epilepsy, the seizure, can be made at the level of single brain area through electrocorticography or of multiple brain areas through the spatial filtering due to the intervening skull, scalp and skin when doing electroencephalography [13]. Above that is the clinical manifestation of alterations of behavior seen in convulsions as well as the more subtle alterations of cognition that are noted in the interictal state [14].

Targeted drug discovery, and rational pharmacotherapeutics, has thus far primarily referred to methods for designing ligands to target specific receptors identified by prior therapeutic experience or experiment. The next level of drug discovery through rational exploration will add the use of these mechanistic multiscale computer models to identify receptors or other proteins to be targeted. It has been suggested that 'The application of [computational] systems biology to medical practice is the future of medicine.' [3] Compared to other brain diseases, epilepsy is the ideal disease substrate for these advances: (1) biomarkers are available – seizures can be identified by EEG; (2) many of the known drugs for epilepsy act on voltage- or ligand-sensitive ion channels, thereby providing an pre-identified set of parameters to consider as drug targets (3) polypharmacy and multitarget pharmacy from multitarget drugs are common in epilepsy and provide a level of complexity that cannot be understood without explicit computational models (4) seizures were the first disease manifestation that were described with explicit multiscale simulation, having been studied in this way for 40 years [4,5]. (5) Recent advances in biological measurement and in computational methods make possible ever larger and more accurate simulations.

The multifactorial causation of epilepsy, exemplified in the river metaphor, can best be approached by computer models that are able to encapsulate the many conspiring and counteracting causes and mitigating or exacerbating influences [1]. Though it is possible to experimentally determine and then conceptualize how a single mutation could produce seizures, modeling is required in order to understand how 2, 5 or 10 such mutations could lead to seizures where none would alone. This complexity also extends to the therapeutic domain, where many drugs are noted to have multiple binding sites and multiple effects. This complexity has traditionally been downplayed by calling the drugs 'dirty drugs,' in presumption that the additional binding is likely to be a cause of undesirable side effects while a single primary binding site is responsible for the therapeutic effect. This leads pharmacologists to attempt to achieve ever-greater ligand specificity in an effort to avoid these multiple effects. However, in some cases, this 'dirtiness' – the binding and activation across multiple different receptors, may be a critical aspect of the drug's efficacy [6–8]. Development of anticonvulsant drugs will benefit from an understanding of how these multiple effects can be synergistic. Perversely, there may be cases

where modern highly-selective drugs might then best be used in combination with other highly-selective drugs, to recreate the multi-target effect that had been so carefully eliminated from individual agents.

Conclusion

Computational modeling of the brain is the best way to get a handle on the complexities of the use of multi-target polypharmacy as well as the complexities of current single drugs that affect multiple sites. One current confusion in the pharmacology of anti-epileptic drugs arises from the effort to identify one drug effect as primary and the other drug effects as either irrelevant or actively harmful through causing side effects. Modeling will enable us to see how and when these effects may be synergistic, contributing together to the reduction of seizures. This perspective seems reasonable when one considers that most and likely all physiological signaling agents are active at different receptor subtypes with different effects.

Conflict of interest

The author has no conflict of interest to declare.

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Computational Models of Neurological Disorder

Computational modeling to advance deep brain stimulation for the treatment of Parkinson's disease

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Deep brain stimulation is effective at improving motor symptoms of Parkinson's disease. However, the mechanism of action remains unclear and more efficient approaches to stimulation may improve patient quality of life. Here we review how computational models have been used to understand and advance the therapy. We describe two classes of models: (1) abstract models, which aim to replicate behaviors without simulating exact patient measures, and (2) clinically predictive models, which aim to simulate patient specific parameters. Abstract models can be used to develop novel patterns of stimulation while clinically predictive models can be used to aid clinicians in selecting therapeutic stimulation parameters for each patient. These principles can likely be applied to stimulation therapies for a number of disorders.

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Introduction

When pharmacological treatment is either not effective or produces debilitating side effects, deep brain stimulation (DBS) has proven to be an effective therapy for many neurological disorders, including Parkinson's disease (PD), essential tremor, epilepsy, and obsessive compulsive disorder [1]. While electrical stimulation may be more focal than many systemically delivered drugs, the effects of electrical current on neural tissue and network dynamics can be complex and the mechanisms can be difficult to understand. Computational modeling can be used to help interpret experimental findings, provide insight into potential mechanisms of action, and provide a platform for developing and testing novel approaches for stimulation therapies. In this article we will focus on examples of how computational models have been used to understand and advance deep brain stimulation for Parkinson's disease.

Computational modeling has been impactful for improving DBS for treatment of motor symptoms of PD for several reasons. First, there is a brain network hypothesized to be responsible for motor symptoms of PD and that is the target of DBS therapy. A loss of dopaminergic input to the basal ganglia thalamo-cortical network leads to impaired motor function. DBS involves implanting electrodes into specific targets within the basal ganglia and continuously delivering electrical pulses. Having a discrete network thought to be responsible for the pathology provides a well-defined system that can modeled where each component is based on physi-

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ologically identified parameters. Second, while DBS is therapeutically effective, the mechanism of action is unknown. There are, however, a number of experimental findings about how the system acts in response to stimulation. Finally, there is a need for improved stimulation and optimization approaches. Currently, high frequency (>100 Hz) square wave stimulus pulses are delivered continuously. This can result in negative side effects, such as cognitive and speech impairments [2], and does not adapt to changes in the physiology. Improved efficacy and reduced side effects may be achieved through improved stimulation methods. Computational models offer a platform to develop and test novel DBS algorithms and move towards patient-specific therapies.

When a therapy, such as DBS, is not well understood, computational models characterizing experimental findings provide a way to formalize a hypothesis of how the therapy might work. Writing down equations to represent components of the system can help identify all the parameters of the system that are important and how they interact. Developing a model facilitates our understanding of: (1) how sensitive the behavior of the model is to particular coefficients, (2) whether the heuristic understanding of how components of the system interact can actually produce the expected behaviors, and (3) whether all necessary components of the system are actually identified and well characterized by physiological experiments. Failure of a model to reproduce the desired behavior is actually the most informative because it identifies that the heuristic working model is probably incorrect and needs to be revised.

Here we will present two different ways computational models have been used to understand and design stimulation therapies. The first is to use of more abstract computational models to understand mechanisms of a therapy and help develop and test novel approaches. The second is to use a computational model simulating patient specific measures to aid a clinician in optimizing stimulation parameters for the patient. Abstract models do not attempt to simulate exact patient specific measures, but instead aim to replicate physiological behaviors to provide an understanding of how stimulation parameters, such as waveform shape, may affect the behavior. These models allow for experiments to be performed virtually that may be difficult, or even impossible, to do in an *in vitro* or *in vivo* experimental model. The goal is not to identify the exact stimulation parameters that would be taken to the clinic, but to understand the mechanism of stimulation and use the model to predict an approach that could potentially more efficiently target pathological activity. Alternatively, clinically predictive models attempt to simulate patient specific physiological parameters in the most realistic way possible. In a clinic where the physician must choose stimulation parameters, MRI or CT scans can be used to generate an accurate model accounting to describe the volume of tissue activated using various parameter settings to help identify stimulation parameters best suited to the patient. Patient specific modeling can support the clinician in tuning a therapy by reducing the guesswork and providing computationally optimized parameters. We will address abstract models and patient specific models in detail in the following sections.

Abstract models

Computational models must always make certain assumptions. Reducing the model to the minimum necessary components improves computational efficiency, minimizes the free parameters, and facilitates analytic approaches to understand the behavior of the model, all of which help us gain information which can be difficult to obtain in a biological environment. It is often assumed that with enough coefficients a model can reproduce any behavior. However, when each component of the model is informed by physiology, the model is highly constrained, and it may not be trivial to reproduce the desired behaviors. In this section we discuss how models constrained by biology, but not specific patient detail, have been useful in understanding and improving DBS for PD.

In PD, the loss of dopaminergic input to the basal ganglia leads to a number of changes in activity throughout the network, some of which may appear paradoxical. This includes changes in the rate and pattern of spiking activity and the emergence of enhanced synchrony [3], enhanced coherence between populations [4], and enhanced coherence between frequency bands [5]. Stimulation has been found to disrupt or mask this pathological activity, but this is not necessarily achieved by restoring the neural activity to that seen in the healthy state (i.e., [6–8]). Computational models of the network can be used to understand how stimulation interacts with neural activity to alter network function, providing insight into how DBS may work and how to more efficiently target pathological activity.

Computational models to develop mechanistic insight

DBS was developed as a reversible alternative to ablative surgery [9]. Because stimulation had the same behavioral outcome as tissue removal, it was initially thought that DBS works by inhibiting activity within the target structure [10]. However, experimentally it was found that while stimulation may inhibit local neural activity, it can excite downstream targets [11]. A computational model of the electric field and its effects on surrounding neurons and axons was used to show that DBS can simultaneously suppress activity in the soma while exciting axons [12,13]. This demonstrates how models can be used to explain complex experimental findings and offer insight into the effects of stimulation on the surrounding neural tissue.

An alternative hypothesis to the suppression of neural activity is that DBS regularizes neuronal spiking activity [14]. The Rubin–Terman model was one of the first spiking neuronal network models of the subthalamopallidal network to simulate DBS [15]. In this model they used a network of Hodgkin–Huxley style neurons simulating four different cell types: subthalamic nucelus (STN), globus pallidus internus (GPi), globus pallidus externus (GPe), and thalamocortical neurons, with simulated synaptic connections. This model supported the hypothesis that DBS regularizes firing by driving spiking which unblocks signals passing between the cortex and thalamus. The Rubin–Terman model has been used in many investigations to study cellular effects of stimulation [16–18]. Other models, similar to the Rubin–Terman but incorporating more biological detail, have been used to further investigate how stimulation modulates spiking rate [19,20], the amount of bursting activity [21,22], action-selection [23], and firing pattern [24–26].

Novel stimulation approaches designed in computational models

Therapeutic DBS involves continuously delivering high frequency periodic stimulation. While effective, this approach can lead to a number of unwanted side effects, including cognitive and speech impairments [2]. Designing stimulation approaches to more efficiently deliver stimulation has the potential to improve patient quality of life by reducing negative side effects and improving efficacy. Furthermore, a more efficient pattern of stimulation may be able to use less battery power, thereby reducing battery replacement surgeries for patients. A number of different novel stimulus patterns and approaches have been developed with the use of computational models.

Computational models simulating a potential mechanism of action can be used to design more efficient stimulation patterns that maximize the effect of stimulation while minimizing stimulation energy. One hypothesis is that DBS works by desynchronizing neuronal populations that produce emergent pathological synchrony. Peter Tass used an abstract model of coupled phase oscillators simulating synchronous neurons to develop a novel stimulation approach to specifically and efficiently target enhanced synchrony. The approach, called Coordinated Reset [27], uses stimulation through multiple electrodes along the DBS lead to entrain subpopulations of neurons to each electrode, thereby desynchronizing the overall population [27,28]. After developing and testing the theory in computational models, the approach was tested in a parkinsonian non-human primate model [29,30] and patients with PD [31], where efficacy was shown to last many days beyond the termination of stimulation. Using a similar model we proposed that DBS desynchronizes neuronal populations through ''chaotic desynchronization'', where neuronal responses to the stimulus are sufficiently different that the neurons desynchronize [32]. This model could explain why some stimulation frequencies are more effective than others. Assuming this mechanism, we are able to optimize stimulus frequency and waveforms to disrupt enhanced synchrony using a single electrode [33,34].

Models of various physiological detail may be used to design stimulus patterns that disrupt or mask neural biomarkers of PD. Warren Grill utilized a biophysical spiking network model of the basal ganglia and thalamus to design a temporally optimized pattern of stimulation more energy efficient than high frequency periodic stimulation [35]. A genetic algorithm was used to select an optimal stimulus pattern of lower frequency through many iterations of applying different patterns to the model and evaluating the effects of each on information flow through the network. When tested in a parkinsonian rodent model, and then in patients with PD, the temporally optimized stimulation pattern with lower stimulation frequency was found to be as effective as high frequency stimulation but used roughly 30% of the energy. Furthermore, the optimized stimulation pattern also reduced the enhanced oscillatory activity seen in PD, even when this was not explicitly accounted for in the optimization. The general network model used in this study was accurate enough to produce a pattern that was shown to be effective in multiple subjects; however, it is possible that efficacy may be improved by tuning the model to reflect each patient's physiology.

Computational models for testing closed-loop approaches to stimulation

Optimal stimulation parameters may be unique for each patient. Developing a computational model with sufficient accuracy to design a stimulation pattern to be predictive for all patients may be impossible. Instead, a closed-loop approach, where subject specific responses to stimulation is used as feedback, can be used to optimize stimulus parameters and patterns. Closed-loop algorithms for tuning stimulus parameters based on patient physiology could help more systematically and efficiently identify optimal settings. Computational models can act as a virtual patient, used as a platform test whether the algorithm is able to identify stimulus parameters or patterns to optimally disrupt neurological biomarkers characterized by the model. While optimizing DBS parameters in the model may not result in exact parameters to translate to the patient, the computational model provides a platform to quickly and efficiently test and identify potential algorithms before moving to the clinic. Testing these adaptive algorithms in a computational model, where the optimal stimulation parameters can be identified through brute force search of parameter space, allows for validation of the algorithm outcomes.

One approach for tuning stimulus parameters, such as frequency and amplitude, is to use a machine learning algorithm to optimize stimulation parameters online. Algorithms such as reinforcement learning [36], gradient descent [37], and response surface methodology [38] are approaches widely used in system process engineering for optimizing chemical reactions and other industrial processes. As these approaches have not yet been widely used in adaptive therapies, computational models are an ideal platform to first test and develop adaptive closed loop algorithms before applying them to patients or even animals.

Clinically predictive models

Models can be used to aid clinicians in predicting patient specific outcomes to DBS and to understand variability of outcomes across patients. Fitting a model to clinical data, such as anatomical MRI or CT scans, can be used to model the volume of tissue activation (VTA) produced by stimulation, provided the location of the electrode and the anatomy of the surrounding tissue is known. These models correlated with clinical outcomes can be used to help guide future DBS settings and lead design.

Computational models to predict volume of tissue activation

The effects of electrical current on the tissue surrounding the DBS lead are complex and still not fully understood. Finite element models (FEM) can be used to predict how electric fields penetrate the tissue [39]. However, stimulation can have different effects on cell bodies and the axon fiber bundles near the electrode. Generating a finite element model from imaging data and pairing it with physiologically realistic neuronal models can be used to accurately predict the volume of tissue activation [40]. These models coupled with patient outcomes can help identify the spatial area and type of tissue that must both be activated to correlate with symptom improvement [41–45] and avoided to prevent adverse side effects [46]. These types of models are continuously being updated to include further biological detail and used to understand what level of complexity is needed to adequately capture experimental findings [47].

Optimizing stimulus parameters

DBS is currently tuned without taking into consideration how stimulus parameters affect the spread of the electric field. Cicerone is a modeling tool that predicts VTA to aid a clinician in visualizing the effects of various stimulus parameters on the surrounding anatomy [48,49]. Tuning stimulation parameters with Cicerone was equally as effective at improving motor symptoms as clinical tuning, however adverse cognitive side effects were reduced by using the software to select stimulus parameters which avoided spread of the electric field to non-motor areas of the stimulation target [46].

Novel lead design with computational models

The volume of tissue activated is dependent on physical properties of the electrode as well as stimulation parameters [13,39]. DBS leads currently used clinically feature a cylindrical shape with 4–8 contacts wrapping around the lead, equally spaced apart along the length of the lead. New electrodes that segment the ring electrodes around the lead are being designed to allow the current to be steered [50]. Computational modeling has shown current steering using segmented leads may be beneficial in focusing the stimulation when the electrode is placed slightly off target or to target brain regions with complex geometries [41].

Many leads are in the process of being developed to allow for current steering, ranging from St. Jude's 1-3-3-1 lead (St. Jude Medical, St. Paul. Minnesota), where the center electrodes are divided into 3 segments, to the Sapiens lead (Medtronic, Minneapolis, Minnesota) with 40 contacts. Segmenting the lead is a tradeoff between improving the ability to steer the current and maximizing surface area of the electrodes. Computational models have been used to inform future device design by investigating the optimal number of segments along and around the electrode [51].

As more complex electrode geometries are developed, the parameter space for stimulus design increases, requiring more guidance for selecting stimulus parameters. Different approaches have been developed in FEM models to help identify optimal stimulus contacts, such as a machine learning approach [52] and a particle swarm approach [53]. Computational models can continue to be used to inform optimization approaches for selecting the optimal combination of contacts to steer electrical current to specifically activate targets which correlate with motor improvement.

Conclusions

For computational modeling to advance a therapy there needs to be, (1) a well-defined system in which the pathology occurs, (2) a hypothesis of how stimulation modulates the neural activity, and (3) good biomarkers that can be used to assess the therapeutic outcome. Computational models help explain experimental findings and formalize our understanding of underlying mechanisms, guide the next generation of device design, provide a platform for developing and testing promising novel stimulation approaches, and aide clinicians in tuning stimulation parameters.

Stimulation and device design used in DBS for Parkinson's disease was relatively unchanged for the first 20 years of the therapy. Computational modeling has in part fueled an emergence of multiple new promising approaches and advances over the past 10 years. The success of DBS for PD has led to its application to more than 30 other clinical disorders, particularly in patient populations who are poorly controlled with pharmaceuticals alone [54]. In addition to DBS, electrical stimulation therapies are used to target nerves,

such as vagal nerve stimulation for epilepsy [55] or sacral nerve stimulation for urinary incontinence [56]. Furthermore, there is increased interest in understanding the mechanisms and optimization of non-invasive stimulation therapies, such as transcranial direct current stimulation (tDCS), transcutaneous electrical nerve stimulation (TENS), and Transcranial Magnetic Stimulation (TMS). While the benefits of stimulation in some disorders, such as PD, are clear and robust, clinical trials for other applications, such as major depression, have had some failures [57]. It may be that in these disorders effective stimulation parameter windows are smaller and non-overlapping between patients. Use of computational modeling may be a necessary component of optimizing the therapy to achieve reliable clinical outcomes.

Computational models will continue to develop and be more physiologically realistic. Projects such as the Human Brain Project [58] and Blue Brain Project [59] aim to make a computer simulation of the entire brain to enhance our understanding and advance brain related medicine. In the future, these platforms may allow for testing therapies, both pharmaceutical and stimulation, in a computational intensive, but biologically realistic environment before moving to patients. Computational models have facilitated our understanding of the mechanisms underlying deep brain stimulation and have helped drive new therapeutic approaches. There are many opportunities to generalize this approach to optimize DBS for other diseases and neuromodulation with other modalities.

Conflict of interest

T.I.N. consults for Medtronic (Minneapolis, MN). A.B.H. has no conflict of interest to declare.

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Computational Models of Neurological Disorder

Capturing intracellular Ca^{2+} dynamics in computational models of neurodegenerative diseases

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Many signaling pathways crucial for homeostatic regulation, synaptic plasticity, apoptosis and immune response depend on Ca^{2+}. Ca^{2+} dysregulation disrupts normal function of neurons and neuronal networks. This causes severe motor and cognitive disabilities. Understanding how Ca^{2+} dysregulation triggers disease onset and progression, and affects downstream processes, can help identify targets for treatments. Because of intermingling of molecular pathways, dissecting the role of individual mechanisms and establishing causality is very challenging. Computational models provide a way to decipher these processes. I review some computational models with Ca^{2+} dynamics to illustrate their predictive power, and note where extending those models to capture multiscale interaction of Ca^{2+} dependent molecular pathways can be useful for therapeutic and drug discovery purposes.

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Introduction

Proper handling of intracellular Ca^{2+} is essential for excitability, homeostatic regulation, synaptic plasticity, pathogenesis and apoptosis. A complex network of Ca^{2+} regulatory machinery maintains the cytosolic Ca^{2+} levels within a physiological range over the life span of the neuron. Improper handling of Ca^{2+} can destabilize the homeostatic process, alter the network connectivity patterns, and can eventually lead to neuronal death. Although the malfunctioning of Ca^{2+} regulatory mechanisms is typically observed in all of the neurodegenerative disorders, the process that triggers the disease onset and cause malfunctioning of Ca^{2+} regulatory mechanisms is not understood. Computational models with Ca^{2+} regulatory mechanisms and Ca^{2+}-dependent signaling pathways can help establish the basis of disease onset and progression and eventually could allow uncovering targets for drug delivery. Here I review a few computational models of neurodegenerative diseases with emphasis on Ca^{2+} dynamics and argue how those models can be extended to be used for treatment of neurodegenerative diseases.

Ca^{2+} dysregulation in neurodegenerative diseases

The onset of many neurodegenerative diseases is believed to be triggered by abnormal aggregation of intracellular and extracellular toxic proteins such as beta amyloids (Aβ) in

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Alzheimer and α-synuclein in Parkinson. The aggregation of these proteins disturbs the homeostatic regulation by altering the dynamics of Ca^{2+} regulatory mechanisms in healthy cells. In Alzheimer, Aβ could self-aggregate into oligomers, which in turn could then either enhance Ca^{2+} leak from endoplasmic reticulum (ER) into cytosol [1] or enhance mitochondrial-ER cross talk via mitochondria-associated ER membranes (MAMs) [2]. Similarly, α-synuclein in Parkinson may enhance plasma membrane permeability to Ca^{2+}, which could increase Ca^{2+} levels in cytosol and other cytosolic organelles [3]. Extracellularly, Aβ could block K^+ channels and lower the threshold of spiking. This enhances the excitability of the neuron and can cause significant increase in intracellular Ca^{2+} levels [4]. Moreover, protein aggregation and misfolding could directly or indirectly affect the Ca^{2+} permeability of voltage-gated Ca^{2+} channels (VGCCs), AMPARs and NMDARs, or could result in decreased affinity of plasma membrane Ca^{2+}-ATPases (PMCAs), sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCAs), Na^+-Ca^{2+} exchangers (NCXs) and Ca^{2+} buffers. Handling increased loads of Ca^{2+} and breakdown of increased toxic proteins could cause oxidative stress [5] and might trigger autoimmune [6] response leading to homeostatic imbalance, loss of network connectivity and neuronal death.

In Parkinson, dopamine-releasing neurons within substantia nigra (SN-DA) are particularly vulnerable to degeneration compared to other dopaminergic neurons. The Ca^{2+} regulatory mechanisms are not only crucial for dopamine release within a physiological range but also modulate mitochondrial [7] and lysosomal activity [8], their metabolic stress levels, and their vulnerability to degeneration. Because of low levels of Ca^{2+} binding proteins like Calbindin (CB) in SN-DA neurons, Ca^{2+} is mainly removed from cytosol by uptake into ER via SERCA pumps and by mitochondria via mitochondrial Ca^{2+} uniporter (MCU) or mitochondria Ca^{2+}/H^+ exchanger or indirectly via MAMs. This complex intrinsic configuration of Ca^{2+} regulatory mechanisms make SN-DA neurons more vulnerable to any imbalance of Ca^{2+} levels and could easily trigger disease onset.

In Huntington, striatal spiny projection neurons are considered to be the main target for disease onset. In addition to synaptic dysfunction in these neurons, Huntington disease gene (Htt) alters Ca^{2+} homeostatic regulation by disrupting mitochondrial and ER function. Htt directly interacts with the mitochondrial membrane reducing its ability to sequester Ca^{2+} [9]. Bezprozvanny and colleagues showed that mHtt sensitized the Inositol triphosphate receptors (IP_3R1s) to IP_3, thereby enhancing Ca^{2+} release in response to activation of metabotropic glutamate receptors [10]. Subsequent studies indicated that inhibition of IP_3R1 function by genetic knockdown, interfering peptides or pharmacological agents could protect striatal neurons from glutamate-mediated apoptosis [11,12]. In a recent study, Bezprozvanny group showed that striatal dendritic spine loss could also be rescued by inhibition of the store operated Ca^{2+} entry (Ca^{2+} entry through plasma membrane Ca^{2+} channels like transient receptor potential—TRP channels in response to depletion of ER Ca^{2+}), which is observed to be over-active in response to a depletion of ER Ca^{2+} stores because of the higher basal activation of IP_3R1 induced by mutant Htt [13]. Ryanodine receptors (RyRs) mediate Ca^{2+}-induced Ca^{2+} release in response to influx through plasma membrane channels; however, RyR can also act as a source of Ca^{2+} ''leak'' into the cytosol at rest elevating the basal intracellular Ca^{2+} concentration [14]. In neurons, such chronic increases in intracellular Ca^{2+} can alter responsiveness of a large number of signaling pathways, shifting threshold for synaptic plasticity, survival/growth, and other signaling essential for the neurons' role in circuit function.

Mitochondrial dysfunction and alteration in ER-mitochondria crosstalk are thought to be primarily involved in Ca^{2+} dysregulation in ALS affected motor neurons [15] (also see review [16]). Excessive mitochondrial Ca^{2+} accumulation can cause the opening of the mitochondrial permeability transition pore, which has been associated with activation of cell death pathways [17]. It was demonstrated experimentally that altering the physical distance between the opposing membranes affect Ca^{2+} flow from ER to mitochondria and cell viability [18]. The most evident ER abnormality described in ALS is ER stress accompanied by upregulation of the unfolded protein response (UPR). This phenomenon has been amply described in ALS patients [19], as well as in cellular and animal models of ALS [20,21]. However, it is unclear what exactly triggers the activation of UPR. It is likely that proteostasis dysregulation is involved in the activation of UPR. The experimental evidence suggests that MAMs and ER-mitochondria communications, especially lipid metabolism and Ca^{2+} signaling between the two organelles, are logical points of interaction in the pathogenesis of different forms of ALS. Decreased ER-mitochondria interaction could result in insufficient Ca^{2+} transfer from the ER stores to mitochondria and defective bioenergetic coupling. It could also alter the autophagic process, because of impaired vesicle biosynthesis. However, abnormally increased or persistent ER-mitochondria contact might result in enhanced Ca^{2+} flux into mitochondria, triggering mitochondrial permeability transition and apoptosis. RyRs could also be potentially dysregulated in ALS causing abnormal cytosolic and mitochondrial Ca^{2+} influx [22]. Sensitivity to Ca^{2+} dysregulation may be affected by Ca^{2+} binding proteins, since motor neurons have lower amount of these proteins compared to ALS-spared neurons [23].

Computational models of neurodegenerative disorders with Ca^{2+} dynamics

As described in previous section, it is evident that the impact of neurodegenerative disorders on Ca^{2+} dysregulation is

multiscale. However, most of the computational models of neurodegenerative diseases developed in the past considered a single factor. For example, some models only focused on understanding the dynamics of proteins aggregation underlying neurodegeneration [24–27], whereas the others focused on understanding how an individual subcellular organelle and its associated pathways function under normal and diseased condition [28]. The dynamics of Ca^{2+}, which mediates disease onset, disease propagation and control homeostatic regulation, signaling pathways and apoptosis, were either completely ignored or included with minimal details. At network level, the mechanisms of synaptic dysregulation and their effects on network connectivity and homeostatic regulation were only explored at phenomenological level [29–31].

Although those single-factor models provide useful insights into brain disease, the pathological consequences necessarily end up being attributed to a single factor that was picked out to be explored in the model. Such self-confirmatory results are not incorrect; typically the factor being explored *is* of importance. However, as noted above neurodegenerative diseases cause disruption of many interacting processes. Therefore, detailed multiscale mechanistic computational models of neurodegenerative diseases with excitability, detailed dynamics of Ca^{2+} regulatory mechanism and Ca^{2+} dependent signaling pathways would be more useful. A few computational models of neurodegenerative diseases with Ca^{2+} dynamics captured at different scales are briefly described below. These examples show how simple computational methods can be used to understand the disease mechanisms and if extended to include more details can provide a powerful tool for therapeutic and drug discovery purposes.

An example of a computational model of protein aggregation was developed by De Caluwe and Dupont [32]. Their model qualitatively described the interactions between intracellular Ca^{2+} and Aβ. The rise and decay of intracellular Ca^{2+} and Aβ level were captured by a single rate constant for each. The activation of Aβ synthesis by Ca^{2+} was represented by a Hill term with a maximal rate V_α, half-saturation constant K_α, and a Hill coefficient n. They also considered that Aβ oligomers induce Ca^{2+} entry into the cell, putatively by provoking an increase in plasma membrane permeability. This process was characterized by a cooperativity coefficient m, and a rate constant k_β. Using that model the authors showed that a 'steady state' characterized by low levels of Ca^{2+} and amyloids, coexist with 'pathological state' where the levels of both compounds are high. Thus, a large enough perturbation in either amyloid metabolism or up regulation of Ca^{2+} homeostasis could trigger AD onset.

Both experimental and computational modeling studies have shown how Aβ-mediated alteration of Ca^{2+} regulating mechanisms can cause increased cytosolic Ca^{2+} levels in patients with Alzheimer's disease. For example, Good et al. [4] showed in cultured hippocampal neurons that Aβ could block fast inactivating K^+ currents without affecting its kinetics. Later Good and Murphy [33] used a mathematical model of hippocampal neuron to show that Aβ-mediated block of A current could result in increased intracellular Ca^{2+} levels and increased membrane excitability. In their model, they used an immobile Ca^{2+} buffer with a single binding site, a Ca^{2+} extrusion pump (using Michaelis–Menten kinetics), Ca^{2+} diffusion and several VGCCs to regulate intracellular Ca^{2+} levels. They also showed that an increase in Ca^{2+} buffering capacity or decrease in density of VGCCs could reduce the Aβ-mediated effects causing less increased Ca^{2+} levels and excitability.

In another study, Morse et al. [34] used a multi-compartment computational model of hippocampal pyramidal neuron to show that oblique apical dendrites are more vulnerable to Aβ during back propagating action potential because of their proximity to the axo-somatic region in contrast to apical tuft dendrites and the smaller dendritic diameters. When the excitability of the neuron is enhanced because of the Aβ-mediated block of A-type K^+ channels, the less attenuated back propagating action potential in oblique dendrites may activate larger number of VGCCs. The resulted larger influx of Ca^{2+} into the cytosol can produce much larger concentrations of intracellular Ca^{2+} due to the larger surface-to-volume ration in oblique dendrites [34].

In contrast to these relatively simple biophysicall models, It was only recently that somewhat detailed computer model of a motor neuron linking electrical activity with ATP pathways was constructed by Le Masson and colleagues in a model of amyotropic lateral sclerosis (ALS) [35]. The model included the plasma membrane Na^+/K^+ ATPases, PMCA, SERCA, plasma and mitochondrial NCX, MCU and mitochondrial dynamics for converting ADP into ATP to meet the cell's energy demands. The multi-compartmental neuron model was based on detailed reconstruction of a cat motor neuron, to which a 100 mm unmyelinated axon was added and included I_h, Na^+, K^+, VGCCs and K_{Ca} channels. Using this model, the authors found that a reduction in ATP availability can place motor neurons in a physiological state that leads to prolonged depolarization, massive influx of Ca^{2+}, which ultimately may cause cell death. The authors showed that this process involved a positive feedback loop in which small deficits in available ATP lead to small ion imbalances that, in turn, caused a higher energy demand on the neuron, which led to worse imbalances. The energy deficit could be localized to the axon terminal and still lead to lethal cascade through a retrograde spread that ultimately reached the entire cell. The study provided theoretical evidences that bioenergetics could be a critical determinant of motor neuron differential susceptibility in ALS. They inferred from their findings that a therapeutic strategy aimed at supporting

bioenergetics might enhance the capacity of motor neurons to withstand pathological insults, thereby prolonging the lifespan of ALS patients.

Mitochondrial dysfunction plays a central role in almost all neurodegenerative diseases. One of the mechanisms involved in this dysfunction is MAM, which primarily facilitates ER-mitochondria cross talk. It is known that aggregated proteins such as Aβ and α-synuclein alter the dynamics of MAMs, therefore including MAM dynamics in the computational model of diseases will improve understanding of the emergent behavior of the interactions of several dysfunctional Ca^{2+} regulatory mechanisms. Szopa and colleagues developed a model of ER-mitochondria cross talk to investigate its effects on Ca^{2+} oscillations [36]. This model was extended from an existing model [37] to capture Ca^{2+} regulation by ER, mitochondria and the interaction between ER and mitochondria. The Ca^{2+} influx into mitochondria was modeled via mitochondrial Ca^{2+} uniporters (MCU) located in MAMs sensing elevated Ca^{2+} concentrations in ER and Ca^{2+} uniporter located outside MAMs sensing cytosolic Ca^{2+} levels. The Ca^{2+} release from mitochondria into cytosol was represented by Na^{+}/Ca^{2+} and H^{+}/Ca^{2+} exchangers with the rate of Ca^{2+} efflux regulated by the cytosolic Ca^{2+} concentrations. The Ca^{2+} influx into ER was modeled using a SERCA pump whereas the Ca^{2+} release into the cytosol from ER was modeled using a Ca^{2+} leak channel and IP_3Rs/RyRs. A single Ca^{2+} binding protein was included in the model to bind free cytosolic Ca^{2+}. The model produced Ca^{2+} oscillations where the period of oscillations depended on the Ca^{2+} permeability through MAMs. The model predicted that for sufficiently large Ca^{2+} permeability the oscillations disappeared, resulting into high Ca^{2+} levels in mitochondria, which could trigger the early steps of an apoptotic pathway.

Applications to drug discovery

The computational models of neurodegenerative diseases described in the previous section have been helpful in providing mechanistic explanation of neuronal malfunctioning in disease states. Similarly, these models could be used to identify the drugs and their targets for reducing degeneration or rescuing neuronal functioning. For example, let us consider a computer model of Alzheimer disease in which the effect of Aβ-induced block of K^{+} channels is simulated by reducing K^{+} channel density. Here, elevation in Ca^{2+} levels is caused by neuronal hyperexcitability. We can use such a model to identify target ion channels (e.g., VGCC), which can be blocked using specific drugs to rescue the excitability of the affected neuron back to the normal range to prevent Ca^{2+} buildup and allow downstream signaling pathways to function properly. Using the dose-response curve for a potential drug and its specificity in blocking a particular ion channel, we can incorporate dose-dependent modulation effects on ion channels into the model and suggest potential treatments. However, we must be aware that our models will only show how to prevent abnormal neuron firing but not take account of how homeostatic regulatory mechanisms, not included in the model, will respond to the intervention. This is a major concern because of the many Ca^{2+}-dependent signaling pathways that control transcription and translation. Including accurate details of these multiple levels of homeostatic regulation in computer models is a great challenge for which we don't yet have adequate data or adequate models.

Using computer models to be able to identify drugs for prevention of degenerative disease would require including not only homeostatic mechanisms but also another level of pathophysiological detail. For example, a minimal model would need to implement the dynamics of Aβ production, aggregation, degradation, and its complete interaction with intracellular and extracellular organelles. This would allow us to intervene in the buildup of Aβ plaques by reducing Aβ production from APP using ''secretase inhibitors'' e.g., LY-450139, a gamma-secretase inhibitor, while rescuing the neuronal function and preventing cell death. Other strategies, like inhibiting Aβ aggregation into oligomers by using PBT2, or enhancing removal of Aβ using active vaccines (which produces antibodies to attack Aβ) could be tested using such multiscale computer models of Alzheimer.

Constructing computer models of neurodegenerative diseases with detailed Ca^{2+} dynamics is necessary to link disease dynamics across multiple scales i.e. disease onset, progression, excitability, plasticity, homeostasis and cell death. Such models will not only allow therapeutic intervention at multiple levels, it will also allow to evaluate the effects of treatment across multiple scales and may provide hints on potential side effects. Detailed Ca^{2+} dynamics are needed because neurodegenerative diseases target multiple Ca^{2+} regulatory processes. For example, in Huntington, Ca^{2+} homeostatic regulation is disrupted via mitochondrial and ER malfunctioning, which is caused by enhanced sensitization of IP_3Rs and RyRs. If we construct a computer model of Huntington with detailed Ca^{2+} dynamics including detailed ER structure, ER regulatory channels and pumps, mitochondrial machinery, Ca^{2+} buffers and plasma membrane pumps and exchangers, we could identify multiple strategies to control enhanced Ca^{2+} release into cytosol. For example, treatments could involve partially blocking VGCCs, IP_3Rs, RyRs, pumps and exchangers, or controlling cytosolic Ca^{2+} with buffers. Any such strategy would be expected to prevent Ca^{2+} dysregulation from damaging downstream signaling pathways whose regulation is essential for the proper functioning of neurons and neuronal networks. These techniques could then be used to identify drugs and to provide guidelines for temporal sequences of drug administration. In the case of Huntington, an actual cure the disease would require intervention at genetic level. In other polygenic diseases, the set of

genes might not be intrinsically pathological but would be adequately controlled by medications, as in the case of type 2 diabetes mellitus.

Conclusions

Computational models of neurodegenerative diseases with relatively simple Ca^{2+} dynamics have been helpful in identifying some processes underlying disease propagation, Ca^{2+} dysregulation and synaptic malfunctioning. However, these models must capture more of the detailed dynamics of molecular pathways responsible for the maintenance of neuronal function in order to be of direct use for translational medicine. In particular, current models are limited due to their inability to capture homeostatic mechanisms and the compensatory nature of multiple pathways, which adjust their dynamics in response to pathology. Future models of neurodegenerative disorders will incorporate detailed Ca^{2+} dynamics along with the causal disease factors, and downstream compensatory signaling pathways that work together to produce cognitive effects and motor control.

Conflict of interest

Nothing declared.

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Computational Models of Neurological Disorder

Modeling neurological disease processes using process algebra

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The sheer complexity of pathogenic neurological processes poses a barrier to understanding that impedes the discovery of more effective drugs or drug combinations for the treatment of neurological disorders. Going forward, the principle means of confronting neurological complexity will be computational modeling, and the effort should employ every available tool. Process algebra is a powerful tool developed in computer science for the purpose of analyzing complicated systems. Its recent appearance in computational neuroscience promises to bring new insights into neurological processes. It will be of particular value for *in-silico* screens of drugs and drug combinations, and will allow modelers not only to show that a particular treatment may be effective but also to reveal its potentially complex mechanism of action.

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Introduction

Process algebras are formalisms designed for analysis of data processing procedures [1]. The symbols and operations in process algebras represent the data (numerical or non-numerical) and operations (sequential or concurrent) involved in a process, and this abstraction facilitates representation by a computer. Computationally, process algebras are implemented using declarative programming languages (see next section), and are the tools of choice in computer science for the analysis of complicated processes. Neurological processes are likewise complicated, and deeper understanding could be achieved by modeling them using process algebras as implemented in declarative programs.

Like a program written in a conventional language, a declarative program could be used to simulate a process along a single processing stream, but the process-algebra formalism facilitates analysis of the properties of a process as it continues along many (and possibly all) of its available processing streams. Such capability is essential for computational analysis of neurological disease processes that can proceed concurrently along many separate but interacting pathways. This article will summarize recent efforts to bring process algebra and declarative programming to biology and specifically to neurobiology and neurology. Emphasis will be placed on its use for identification of potentially effective drugs and drug combinations and for explication of their likely mechanisms of action.

Declarative programming

The two basic kinds of programming languages are imperative and declarative. Well known imperative languages include C, Java, and Python. Well known declarative languages

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include Prolog, Lisp, and HTML. The declarative language known as Maude is a mathematical language in which process algebras can be effectively implemented [2–4]. (Maude software and examples are available at: http://maude.cs.illinois.edu/w/index.php?title=The_Maude_System). The overwhelming majority of computational models in neuroscience have been implemented using imperative languages [5]. Declarative languages differ in a fundamental way from imperative languages. While a statement in an imperative language is a command, a statement in a declarative language is a statement of fact (i.e., a ''declaration''). The difference between the two is best illustrated using a simple example.

Consider a system of trails through the woods as mapped out in Fig. 1a. The trail map connects points on the periphery (X, Y, and Z) with intersection points within the woods (C, G, J, M, Q, and T) along trail segments that allow transitions between the points. A program written in an imperative language could command a ''hiker'' to take a specific path through the woods: go from X to T, go from T to C, go from C to Q, etc. An imperative program could also allow the hiker to take a randomly determined path through the woods on each run, constrained by the trail map and perhaps given the further constraints that a hike back to the immediately preceding intersection is not allowed, and that arrival at a peripheral point (X, Y, or Z) ends the hike. With many random trips through the woods the hiker would have followed many different paths but there is no guarantee that the hiker would have followed all possible paths. Consequently, the imperative program could not be used to answer with certainty questions such as: what is the shortest path from X to Y? Or: how many ways are there to hike from X to Z in four segments? Declarative languages were created to answer these sorts of questions and to facilitate analysis of the range of possible behaviors available to a system.

A program to represent hiking in these woods that is written in a declarative language would first specify (i.e., declare) the allowed transitions in the trail system: X goes to T, C goes to Q, M goes to Z, etc. Then running the declarative program could simulate not only one but all possible paths through the woods. The complete transition tree for hikes starting from X to a depth of four transitions, given the same constraints as for the imperative case described above, is shown in Fig. 1b. The transition tree clearly shows that the shortest path from X to Y requires three segments: X to T to G to Y. It also shows that there are exactly four ways to get from X to Z by hiking only four segments.

The properties our simple trail system could be discerned from inspection of the trail map (Fig. 1a) without recourse to the transition tree (Fig. 1b). For more complicated systems it is essentially impossible to discern system properties from inspection of the system diagram; computational analysis of

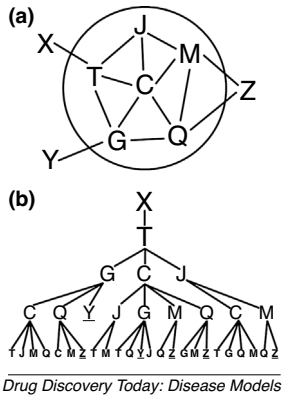

Figure 1. A hypothetical system of trails through the woods (a), and the complete set of hikes that start from X and proceed in four segments (b).
The trail map (a) connects points on the periphery of the woods (X, Y, and Z) with the intersections between trail segments that occur within the woods (C, G, J, M, Q, and T). Hikes proceed according to the following rules: A hiker can move from the current point to any adjoining point except the point immediately preceding the current point. Hikes start from and end at the peripheral points (X, Y, and Z). The transition tree (b) shows all four-segment hikes that start from X and proceed according to the rules. Hike end-points are underlined. The transition tree shows that the shortest hike from X to Y takes three segments, and that there are four ways to get to Z from X in four segments.

the state transition tree is required. Declarative environments facilitate searches of transition trees for specific states and pathways. They also facilitate temporal-logic analysis of transition trees, using which it is possible to prove whether or not certain temporal relationships between specific system states are true [6,7].

Process algebra models of biological processes

The analogy between a trail system (Fig. 1a) and a system of cell-signaling pathways easily extends the idea of a state transition tree (Fig. 1b) to the transitions involved in gene regulatory and developmental processes. Process algebras and related techniques have been applied in the representation and analysis of many kinds of biological systems [8]. Some of these expressly involve specification and analysis of biological processes using declarative programming. Maude has recently been applied in the analysis of biological systems [9]. As a mathematically formal, executable declarative language, models specified in Maude can be used for simulation but also for analysis using state-space search and temporal logic [6,7].

In another recent effort, the declarative language AnsProlog (Answer Set Programming in Logic) was adapted for the analysis of cell signaling pathways [10]. The interactions between signaling pathway elements are represented in AnsProlog in a manner similar to that in which knowledge such as facts and rules are represented in logic-based

programming in general. While this kind of representational scheme has limitations as compared with a mathematically formal, executable declarative language such as Maude, it is capable of powerful reasoning functions that include state-space and temporal-logic analysis but go beyond those to include prediction (state Z will occur), planning (how to get state Z), and explanation (how state Z happened).

Declarative programs that write imperative programs

An advantage of declarative languages is their expressiveness. Rather than use declarative specifications for simulation or analysis, many developers use them to write imperative programs that then implement a simulation of a biological process [11]. For example, a declarative program could specify that a particular enzymatic reaction should occur in the same way for all isoforms of that enzyme (same substrates, cofactors, etc.), except that the reaction rate should depend on the identity of the isoform. Then execution of the declarative program writes an imperative program that repeats the same simulation code for each reaction, except that each enzyme isoform has its own reaction rate.

This approach has been applied to neurobiology. A general framework for using declarative specifications to write imperative programs that simulate neurobiological systems is called NeuroML, which is based on the declarative language XML (Extensible Markup Language) [12,13]. NeuroML supports a wide range of single-neuron formalisms, from integrate-and-fire to morphologically detailed multicompartmental models. NeuroML writes imperative programs that run within well-known neurobiology simulation environments such as Neuron and Genesis. More recent versions even use imperative programs in Python to write large declarative specifications in NeuroML, and then use those to write imperative programs [14].

Using declarative programs to write imperative programs that implement models of neurobiological processes greatly facilitates model creation, especially by groups who may want to run their models within different simulation environments. But this approach does not take advantage of the unique analysis capabilities of process algebras. Other approaches do.

Process algebra models of neurobiological processes

So far, applications of process algebras and declarative programming to neurobiology have been few. One group used process algebra for modeling and analyzing brain development. They wrote rules to act on a topological representation of brain tissue and used that to model the neurulation process, in which a sheet of cells develops into the neural tube [15]. This application involves a declarative language called MGS that has been extended so that rules can operate on data structures called topological collections.

Another group applied declarative programming in the analysis of a central pattern generator (CPG) in *Aplysia* [16]. They wrote a declarative specification of the CPG that can drive several different rhythmic modes of feeding movements in this sea slug. Their effort is notable in that they created an abstract, discrete representation of a neural circuit that is normally represented as an interconnected set of biologically detailed model neurons. Their rationale was twofold. First, existing biologically detailed models include many parameters on which system behavior sensitively depends but whose values are unknown. Their model was composed of ten highly simplified, two-state model neurons (i.e., ''units'') that were all the same and had very few free parameters. Second, the tractability of a simpler model permits not only simulation but also analysis of system behavior. Specifically, this group used temporal-logic analysis to explore the consequences of concurrency in the *Aplysia* CPG model.

The ability not only to simulate but also to analyze models of biological processes is essential because of ''concurrency'', a term originating from computer science to describe the attribute of distributed computing processes by which several separate but interconnected sub-process can occur on their own schedule. Concurrency is also a property of many biological processes. Due to concurrency in the CPG model, the ten two-state units could update in different orders. To perform analysis of the CPG model, it was implemented using a declarative language known as SAL (Symbolic Analysis Laboratory). Temporal-logic analysis was then used to prove key behavioral features of the model, for example, that certain identifiable units had to be active and others inactive before the system could transition from one rhythmic mode to another.

We recently advanced a declarative model of fear conditioning and extinction as observed in rodents [17]. The model diagram, shown in Fig. 2, was adapted from reviews by Joseph LeDoux and co-workers [18,19]. Failure of extinction in rodents is considered to be an important animal model of post-traumatic stress disorder. Normally, an aversive stimulus (unconditioned stimulus, US) elicits a fear response (unconditioned response, UR) from the fear pathway, a neural pathway though the amygdala. The US activates neurons in the lateral amygdala (LA), which relay signals over the fear pathway that ultimately produce the UR by activating neurons in the periaqueductal gray (PAG). The model has two LA units (LA1 and LA2, see below). Fear conditioning involves pairing the US with a previously neutral stimulus (conditioned stimulus, CS), after which the CS can produce the fear response (conditioned response, CR) by itself. Extinction involves the gradual reduction in the CR as the CS is repeatedly presented without the US. Signals related both to the US and CS arrive at the LA from the thalamus and cortex.

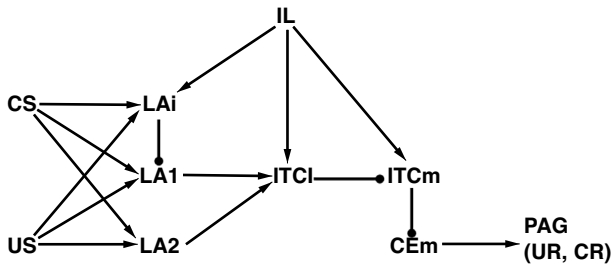

Figure 2. Schematic diagram of the amygdala fear pathway. Neurons in lateral amygdala (LA) receive sensory inputs that naturally produce fear responses or that can be conditioned to do so through conditioning (unconditioned and conditioned stimuli, US and CS). Some (not all) LA neurons also receive input from inhibitory interneurons within LA (LAi inhibits LA1 but not LA2). LA neurons relay signals via lateral and medial sets of intercalated cells (ITCl and ITCm) to the medial part of the central amygdala (CEm), which activates neurons in the periaqueductal gray (PAG) that produce the fear response (unconditioned and conditioned response, UR and CR). The fear pathway through the amygdala can be modulated by neurons in infralimbic cortex (IL) that are active during extinction. Arrow and ball endings represent excitatory and inhibitory synapses, respectively. Diagram reproduced from Ref. [17].

Fear conditioning and extinction involve long-term potentiation and depression (LTP and LTD) of some of the synapses along the amygdala fear pathway, and of connections from the infralimbic (IL) cortex onto inhibitory neurons along the fear pathway. The activity of IL neurons is associated with extinction of fear conditioning. Research using transgenic rodents has shown that extinction also requires the actions of receptors for the endogenous cannabinoids, which cause LTD of the synapses onto LA neurons from inhibitory LA interneurons (LAi in Fig. 2) [20]. This finding poses a paradox. Because LA neurons relay CS signals over the fear pathway in order to elicit the CR, extinction can only occur through decrease in the response of LA neurons, but LTD of the synapses from LAi onto LA neurons would increase rather than decrease LA responses and so suppress rather than promote extinction! The declarative model of extinction was created to resolve this paradox.

To render the model analytically tractable, synaptic strengths were discretized so that each of the 9 adaptable synapses could only take absolute values of 0, 1, or 2. Even the simplified, discretized model of the amygdala fear pathway admits of 3^9 or 19,683 possible synaptic strength configurations. To computationally search this large configuration space, the amygdala fear pathway was modeled using Maude. The program specified the rules for changes due to LTP or LTD that were known to occur for each adaptable synapse during conditioning or extinction. During conditioning, the synapses from CS and from LAi, LA1, or LA2 could each undergo LTP and did so, in all possible orders, until the CS alone could activate PAG and thereby elicit the CR. Then during extinction, the synapses from IL could each undergo LTP, while those from CS and from LAi, LA1, or LA2 could each undergo LTD in all possible orders, until the CS alone could no longer activate PAG. Many different synaptic weight configurations were compatible with conditioning, and many with extinction, and the declarative program was written so that, given the rules for changing synaptic strength, Maude first found all possible synaptic strength configurations that produced fear conditioning, and from each of those found all possible synaptic strength configurations that were compatible with extinction. As an example, the rule that produces LTD of the weight of the synapse from LAi onto LA1 in Maude is:

```
crl [exLAitoLA1]: extinguish(2) US(0) CS(1) IL(1) PAG(1) wLAitoLA1(Y) =>
                  extinguish(2) US(0) CS(1) IL(1) PAG(1) wLAitoLA1(Y + 1)
                  if Y + 1 <= 0 .
```

This rule, labelled exLAitoLA1, is conditional (crl). Whether conditional or unconditional, a rule in Maude replaces a term matching its left-hand side (the term before the => symbol) with a term matching its right-hand side. The operator extinguish takes argument 0 if extinction is not allowed, 1 if extinction is allowed but not yet occurring, or 2 if extinction is occurring. During extinction the US is absent (US(0)) but the CS is present (CS(1)), and IL is active (IL(1)) but PAG (PAG(1)) is still activated by the CS. When these terms are present, then the weight of the inhibitory synapse from LAi to LA1 (wLAitoLA1(Y)) can undergo LTD (i.e., become less negative) on the condition that is does not become positive (if $Y+1<=0$). During extinction, the weights from CS, LA1, and LA2 also undergo LTD while those from IL undergo LTP, and similar rules specify those modifications. The extinction process continues making allowed weight changes until the CS (CS(1)) no longer activates PAG (PAG(0)).

The most interesting feature of extinction is that it is not forgetting, and this is manifested at the neural level by the continued response of some LA neurons to the CS [21]. What this means in terms of the model is that extinction should be associated with continued CS excitation of LA1 or LA2. Maude search found 957 configurations with the following features: they were compatible with extinction, they had LA1 or LA2 excited by CS, and they were associated with LTD of the LAi to LA1 synapse. This shows that extinction with some residual CS response was possible even with LTD of the LAi to LA1 synapse, and raises the question of how many such configurations are *not* associated with LTD of the LAi to LA1 synapse. The answer is 303. The upshot is that LTD of the LAi to LA1 synapse, which opposes extinction, nevertheless triples (303 compared with 957) the number of synaptic strength configurations that achieve extinction while preserving some CS response in LA. This result allows us to speculate that the function of the endocannabinoid system in this context is to allow extinction but prohibit forgetting of

the potential for danger signaled by a stimulus that had at one time been associated with an adverse outcome.

Polypharmacy and process-algebra models of neurological disorders

We have recently applied declarative programming in modeling neurological disease processes, with a focus on Alzheimer Disease (AD). The models concern amyloid-β (Aβ) peptide metabolism, Aβ effects on synaptic plasticity, and Aβ-induced brain inflammation [22–26]. The models of synaptic plasticity impairment and of Aβ metabolism will be summarized here to illustrate process algebra models of AD on two different levels.

Oligomers of Aβ impair synaptic plasticity such that LTP is eliminated and the range of presynaptic activity over which LTD occurs is extended [27,28]. LTD can proceed to the point

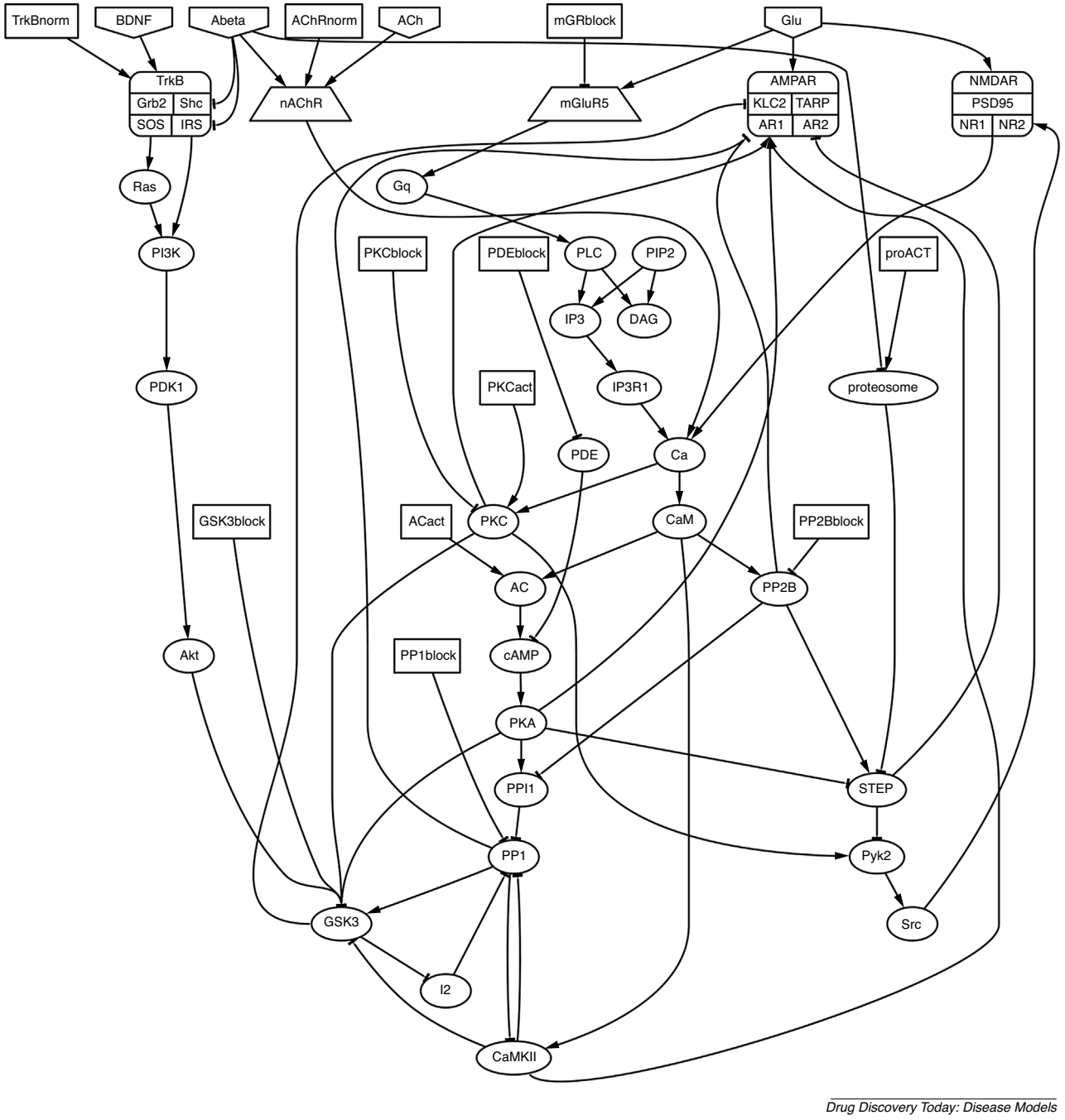

Figure 3. Cell-signaling pathways that mediate synaptic plasticity.
Cell-signaling molecules including kinases, phosphatases, and second messengers are represented as ovals. Receptors are represented as trapezoids or multi-compartment modules, while ligands are represented as pentagons. Drug compounds are represented as rectangles. Arrows and tees represent excitatory or inhibitory connections, respectively. Diagram reproduced from Ref. [24]. See Ref. [24] for all abbreviations.

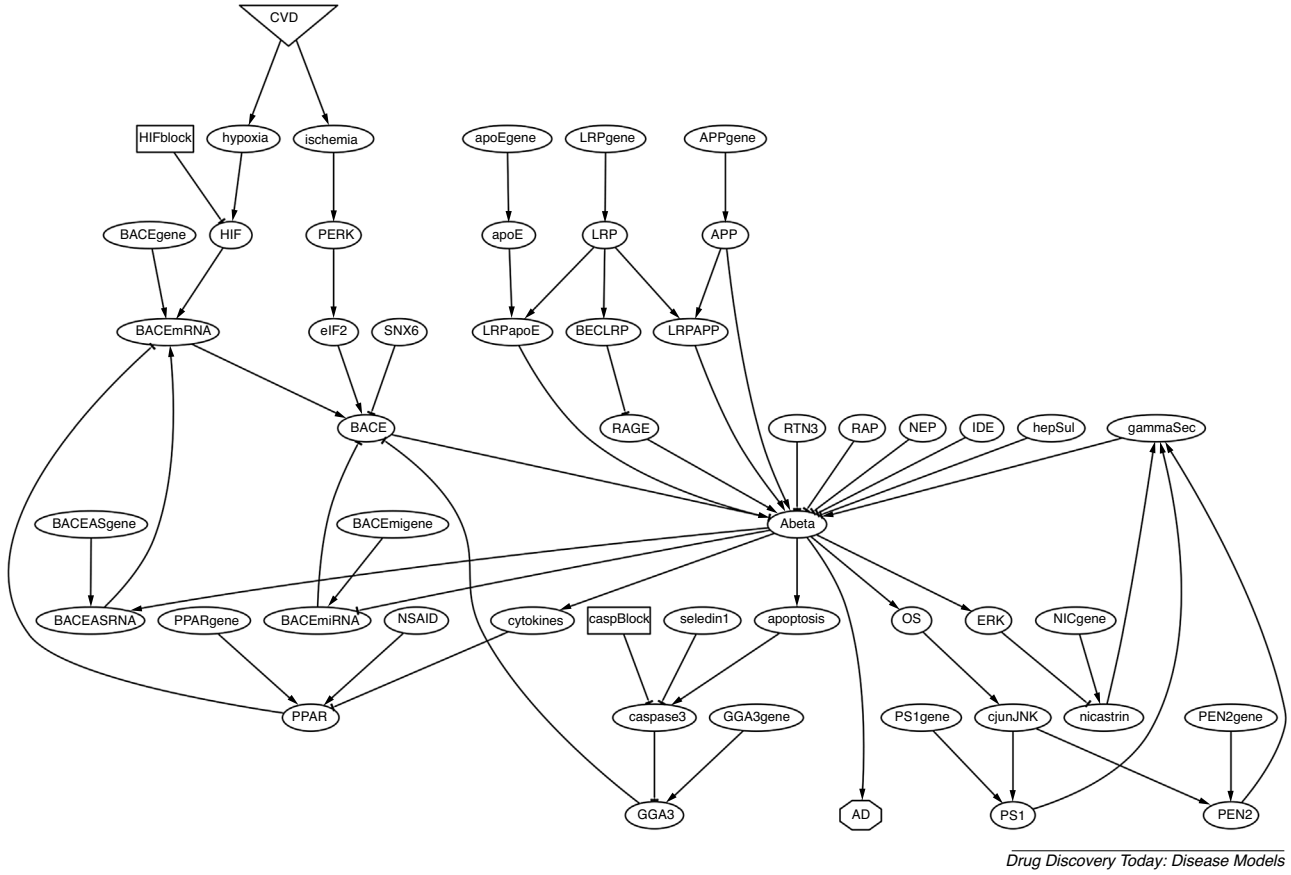

Figure 4. Molecular pathways involved in metabolism of amyloid-β (Aβ).
Regulatory elements include anabolic and catabolic enzymes, signaling molecules, genes, cellular processes, and conditions such as cerebrovascular (CVD; triangle) and Alzheimer (AD; octagon) disease. Drug compounds are represented as rectangles. All other elements are represented as ovals. Arrows and tees represent excitatory or inhibitory connections, respectively. Diagram reproduced from Ref. [22]. See Ref. [22] for all other abbreviations.

where synapses wither, and this is considered to be the beginning of AD neurodegeneration. To better understand this process, the cell-signaling pathways that mediate LTP and LTD were represented in Maude. The model diagram is shown in Fig. 3. Temporal-logic analysis shows how expression of specific kinase/phosphatase patterns are impacted by Aβ such that kinases that mediate LTP cannot be activated, while phosphatases that mediate LTD are activated over an extended range of presynaptic activities. The model suggests several drug combinations that could normalize synaptic plasticity in the presence of Aβ. A particularly effective combination involves pairing a nicotinic acetylcholine receptor agonist with a phosphodiesterase inhibitor.

Aβ would not impair synaptic plasticity if its over-accumulation could be prevented. Unfortunately, attempts to do so using single drugs or antibodies have been notoriously unsuccessful [29]. To better understand this process, the metabolic pathways involved in Aβ regulation were modeled using Maude. The model diagram is shown in Fig. 4. The model includes the enzymes that synthesize Aβ, the β and γ secretases, and the regulation of their expression through various signaling pathways. The model shows how several different factors could serve as triggers for Aβ over-accumulation, including cerebrovascular insufficiency that activates hypoxia-inducible factor α (HIF).

Temporal-logic analysis shows how Aβ could be driven up through a cascade of sub-processes that proceed in a specific order. It also shows how the use of certain compounds, such as a HIF inhibitor, which prevent initiation of early stage sub-processes, can obviate the use of compounds that prevent initiation of sub-processes that occur later in the cascade. The model suggests that pairing a HIF blocker with a non-steroidal anti-inflammatory could reduce Aβ accumulation due to cerebrovascular insufficiency. Such a combination could be a useful prophylactic in cases of trauma or surgery that cause reduced cerebral blood flow, which is a known risk factor for development of late-onset AD [30].

Conclusions

Process algebra, implemented using a declarative language, is a modeling paradigm newly imported from computer science into neuroscience that has recently been applied to neuro-

logical disorders. The declarative modality offers the ability not only to simulate but also to analyze a process. Computational models in general are well suited to *in-silico* screens of drugs and drug combinations. What declarative programming adds is the capability to show not only *that* a combination works but to analyze *how* it works. The advent of process algebra in neurology promises to bring greater understanding of neuropathology and new insights into the design polypharmacy-based treatments for neurological disorders.

Conflict of interest

The author has no conflict of interest to declare.

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Computational Models of Neurological Disorder

Computer modeling for pharmacological treatments for dystonia

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Dystonia is a movement disorder that produces involuntary muscle contractions. Current pharmacological treatments are of limited efficacy. Dystonia, like epilepsy is a disorder involving excessive activity of motor areas including motor cortex and several causal gene mutations have been identified. In order to evaluate potential novel agents for multitarget therapy for dystonia, we have developed a computer model of cortex that includes some of the complex array of molecular interactions that, along with membrane ion channels, control cell excitability.

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Introduction

A number of movement disorders, as well as epilepsy, are associated with increased activity, and likely with hyperexcitability, in cortex. Dystonia is a movement disorder which produces involuntary muscle contractions. It involves pathology in multiple brain areas including basal ganglia, thalamus, cerebellum, and sensory and motor cortices. Although much of the research in dystonia has looked at the role of the basal ganglia, pharmacological treatment is often provided directly to the muscle through injection of botulinum toxin, anticholinergic agents and benzodiazepines. Motor cortex is another possible target for drug therapy, with manifestations that include augmented beta oscillations. Using a mechanistic multiscale model of primary motor cortex, we have assessed parameter combinations that produce dystonia to suggest potential drug combinations that might interfere with these pathological dynamics.

Schematized and mechanistic models for dystonia

Dystonia is a movement disorder that produces intermittent prolonged involuntary muscle activation that results in twisting, turning or posturing of a limb or other body part and repetitive prolonged movements. As with other movement disorders, the difficulty in modeling dystonia stems from the complexity of the motor system itself: the large set of specialized nuclei in brain and spinal cord that are interacting to produce movement in continuous concert with sensory areas in the sensorimotor loop. These areas include basal ganglia,

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thalamus, cerebellum, red nucleus, anterior horn, etc. Even when a primary pathology can be localized to a particular area, plastic responses in other motor and sensory areas will alter the expression of the disease in a way that can either ameliorate or exacerbate disability, and treatments may target areas other than the area of primary pathology. For example, although task-specific focal dystonia such as writers cramp is thought to occur due to overlearning in sensory and motor cortical areas, some of the treatments used are targeting basal ganglia.

The large number of areas involved in motor activity would be best served by simulations that encompass all of these areas. Such an approach requires working out plausible input and output signal patterns for each nucleus or area, and then requires working with highly *schematized models*. Schematized models typically use mean-field approximations, where brain areas are approximated by scalar signals representing overall activity. Some schematized models may include more detailed integrate-and-fire or scalar (perceptron) neural network models [1–3]. However, this intermediate modeling level also lacks the cell and molecular details useful for comparison with pharmacological intervention.

Sanger and Merzenich [4] used a schematized model to identify likely patterns of positive feedback between sensory and motor cortical areas that would lead to runaway excitation. Their cortical control-theory model was able to identify particular dynamical patterns that could potentially be interrupted to prevent the recurrence of these pathological patterns. Interestingly, this provided some suggestion as to the mechanism of self-treatment using 'sensory tricks', where the patient relaxes the dystonia by touching a particular spot – for example, often on the side of the chin to reduce the head-turning of torticollis. However, the limitation of this model, as for other schematized models, was that it could not suggest drugs or drug targets for treatment.

Mechanistic multiscale modeling is an alternative to schematized models that does afford the opportunity to reach down to the molecular scale of pharmacology and thereby assist in the development of novel treatments. These models will include more levels or scales than are included in the schematized model, and for purposes of drug discovery should include some molecular detail.

A mechanistic model of cortical hyperexcitability

Dystonia is a *dynamical disorder* that can be defined by its particular patterns of muscle activation. The excessive muscle activity of dystonia is a consequence of dynamical disorder in brain and spinal cord, associated with higher than normal activity patterns. To the extent that the disease is caused by cortical dysfunction, as assumed by control theory models [4], we identify *hyper-activity* as a manifestation of *hyperexcitability*.

The major disorder of cortical hyperexcitability is epilepsy, manifested by seizures. In both epilepsy and dystonia, underlying causes will include changes or anomalies in ion channel and receptor densities, as well as in cortical wiring [5], which produce excitation/inhibition imbalances and with excessive cortical firing and excessive synchrony [6–10]. The intensity, pattern, and spread of hypersynchrony differ between epilepsy and dystonia. Electroencephalographic signatures of the two disorders also differ, with seizures characterized by powerful discharges that may be time locked to the movement while dystonia shows an increase in beta (12–25 Hz) oscillations [11–13]. In addition to there being various patterns of hyperexcitability in cortex, there are various ways to produce hyperexcitability *in silico*.

We developed a mechanistic multiscale model of cortex (Fig. 1) in which we could identify patterns of activity for: (1) normal; (2) dystonia; (3) epileptiform (seizure) [14]. Model scales ranged from molecular to network so as to permit us to associate potential pharmacological manipulations with alterations in network dynamics. These models therefore combine the domain of computational systems biology – molecular interactions, with the traditional approach to computational neuroscience – models of cells as electrically interacting units with only ion channels represented at the molecular level.

Varying the densities of voltage-sensitive ion channels and receptor densities on pyramidal neurons and interneurons within reasonably ranges resulted in families of models that could be classified as having normal, dystonia-like, or epileptiform activity patterns (Fig. 2). Dystonia models were characterized by synchronous population discharges at beta frequency (~20 Hz). In each case, there were multiple parameter sets that produced similar dynamics [15–18]. This phenomenon is well known in biology where the combinatorics of multiple alleles for every feature, for every ion channel, enzyme and receptor, means that no two people are entirely alike. Despite not being alike, all people show similar dynamics, a phenomenon referred to as parameter degeneracy [19].

We locate particular models that produce dystonia in high-dimensional parameter space. A three-dimensional slice of the eleven-dimensional parameter space (Fig. 3), shown in a normalized space relative to a baseline value, demonstrated that dystonia cases tend to have higher levels of voltage-gated Ca^{2+} channels (L-, N-, T-types; labeled Ca), lower levels of BK K^+ channels in the plasma membrane, and higher levels of ryanodine (RYR) channels in endoplasmic reticulum. In a particular case, we can indicate a direction in parameter space (Fig. 3, arrow) going from a dystonia parameter-set to a normal parameter-set. For any dystonic case in our set, we can identify a simple path, involving one or two parameter changes, which leads to a normal set, indicating alterations to be effected in our simulated 'patient' that would treat the

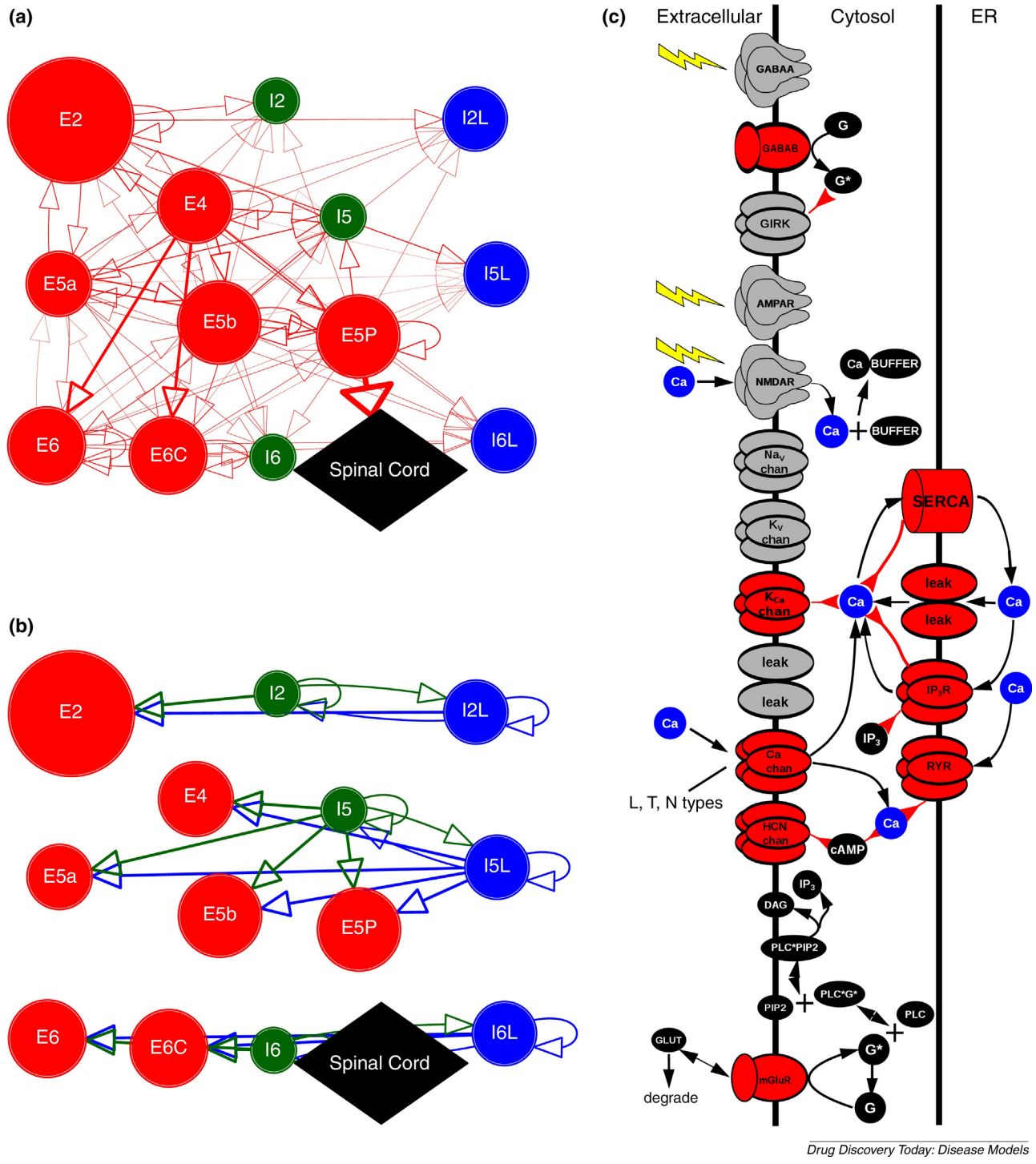

Figure 1. Model schematics. (a,b) Motor cortex architecture. Circles represent neuronal populations (red: excitatory cells; green: fast-spiking interneurons; blue: low-threshold firing interneurons). Circle size denotes number of cells in population. Lines (with arrows) indicate connections between the populations. Thickness of lines proportional to synaptic weights. E cells synapse with AMPAR/NMDARs; I cells synapse with GABAAR/GABABRs. Circles with self-connects denotes recurrent connectivity. **(a)** Excitatory connections. E5P projects to spinal cord (not modeled). **(b)** Inhibitory connections. **(c)** Chemical signaling in pyramidal cells showing fluxes (black arrows) and second- (and third- etc) messenger modulation (red back-beginning arrows). We distinguish membrane-associated ionotropic and metabotropic receptors and ion channels involved in reaction schemes in red (in reality, it is likely that almost every membrane-bound protein is modulated). External events are represented by yellow lightning bolts—there is no extracellular diffusion; the only extracellular reaction is glutamate binding, unbinding, and degradation on mGluR1 after an event. Ca^{2+} is shown redundantly in blue—note that there is only one Ca^{2+} pool for extracellular, 1 pool for cytoplasmic, and 1 pool for ER (PLC, phospholipase C; DAG, diacyl-glycerol; cAMP, cyclic adenosine monophosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate).

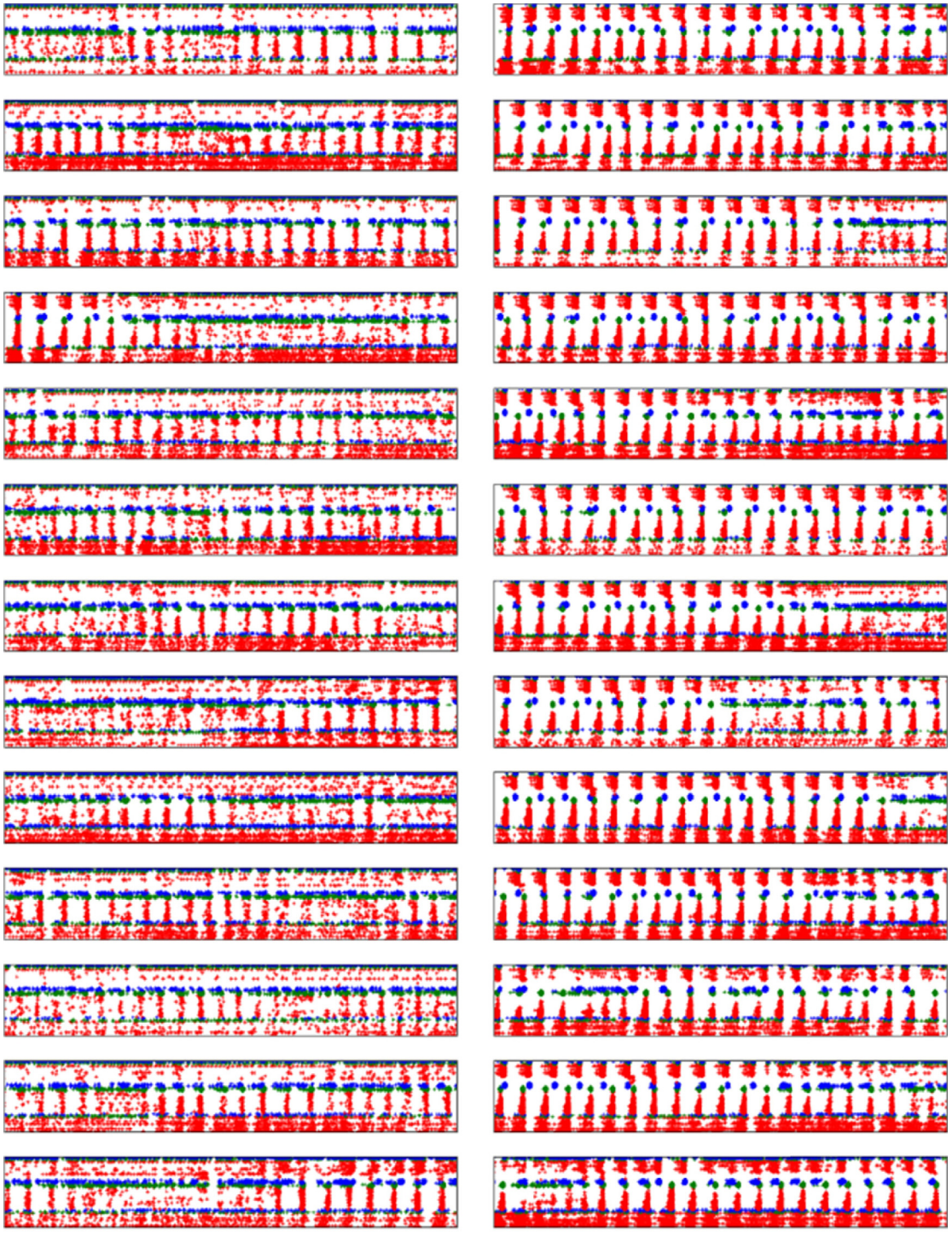

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Figure 2. Raster plot showing 1 s patterns of normal activity (left column) compared to dystonia activity (right column) in multiple cortical models. Red, blue, green dots are from excitatory neurons, low-threshold spiking interneurons, and fast-spiking interneurons respectively. Spikes are arranged by cortical layer (Layers 2/3 at top, layer 6 at bottom). (Within each model, channel densities of neurons of a given type are identical.)

disorder (personalized medicine); the same manipulation would not work for other cases and would therefore not be expected to provide a universal therapeutic approach (similarly we know of many types of dystonias and different gene mutations that can produce hereditary forms). Furthermore, the normal (blue) and dystonia (red) groups do not form well defined clouds. It is difficult to separate normal from dystonic sets, or to separate out different groups of dystonic patients to be treated in a common way. We therefore could not separate out different groups of dystonic patients who could be treated

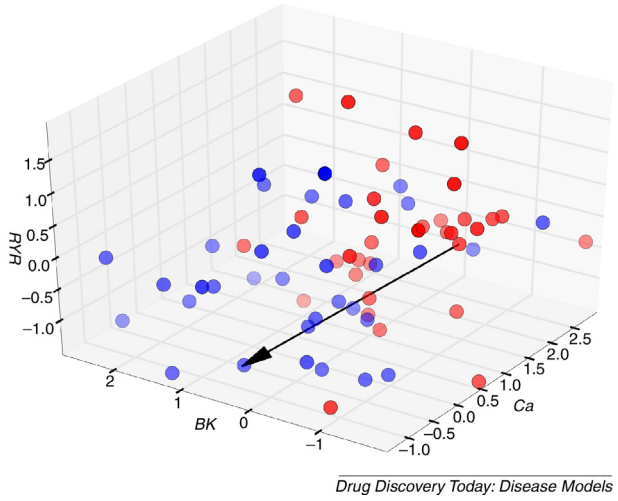

Figure 3. Navigating through three dimensions. Dystonia cases in red and control in blue.

identically within group – precision medicine – despite different treatments between groups.

While it is already difficult to navigate among cases and controls in the three dimensional subspace of Fig. 3, it is impossible to visualize higher dimensions to identify separations between groups in 4 or more dimensions. Instead, machine-learning algorithms are used to identify what is where in high-dimensional parameter spaces. In this case, we first tried an entirely *unsupervised* algorithm, *k*-means, which attempts to find a certain number *k* of galaxy-like clusters of points in the space. Consistent with our difficulties identifying such clusters in 3-space (e.g. Fig. 3), the algorithm failed to separate the data into 2 well-separated groups corresponding to normal and dystonic parameters or, to provide multiple group separations when run with higher *k*.

We then turned to support vector machines (SVM), a *supervised* machine-learning algorithm which separates groups based on user-provided labels – in this case dystonia

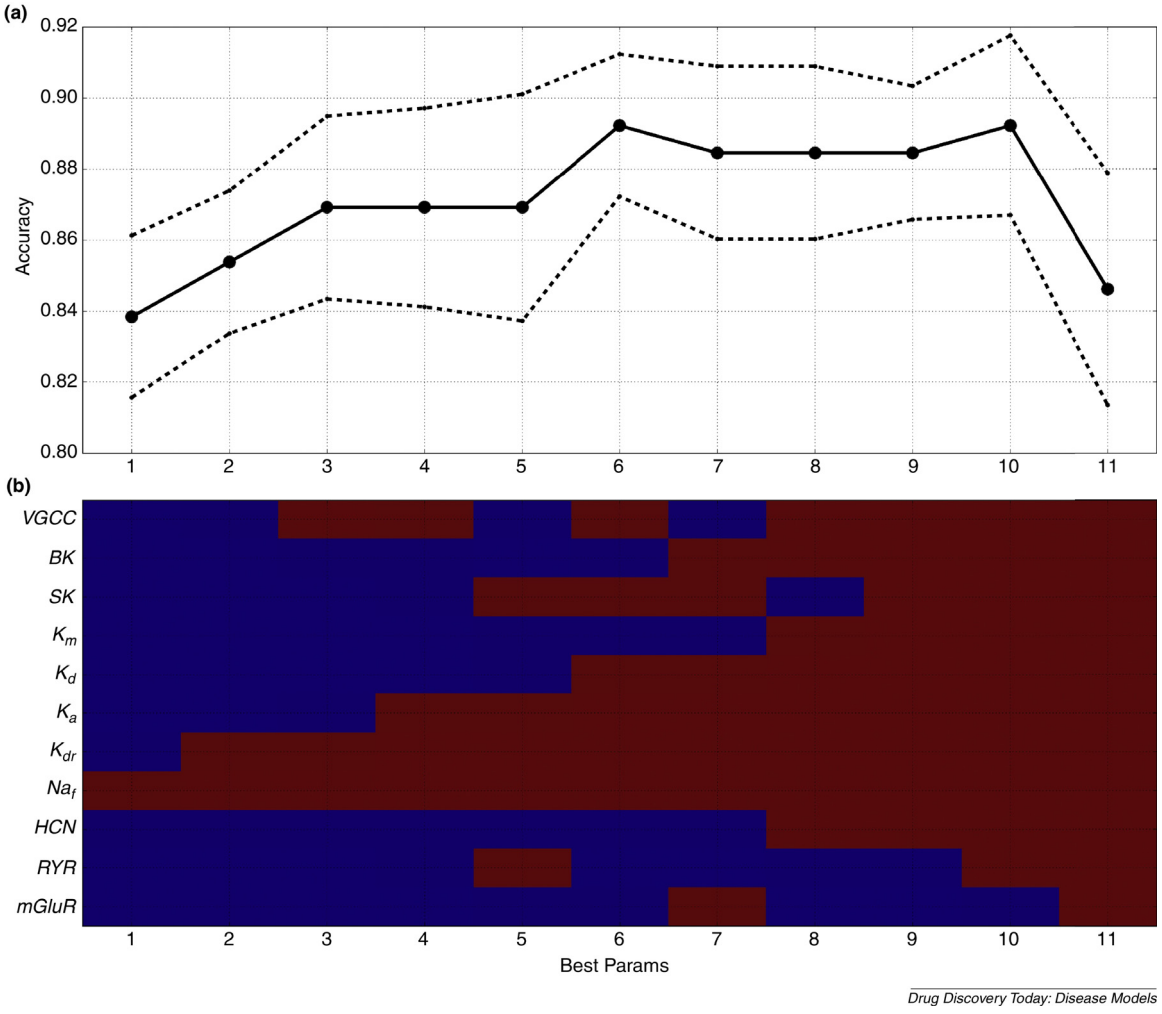

Figure 4. SVM classification accuracy generally increases when using 1–10 parameters, indicating utility of multitarget pharmacy approach to treating dystonia. **(a)** Best classification accuracy from all combinations of *x* parameters (solid line: mean cross-validation accuracy (n = 10); dotted line: standard error). **(b)** Best parameter combinations (red: parameter used; blue: parameter not used). *x*-axis in (a,b) indicates number of parameters used.

vs control. SVMs were run for every potential subspace to determine which combination at that dimensionality gives the best separation. SVMs were able to separate out the 2 groups with a gradual increase in the quality of separation at higher dimensions up to 6 dimensions (Fig. 4). From there, plateauing was seen up to 10 dimensions, likely indicating further improvement in distinction masked by the 'curse of dimensionality' (using a constant number of data points, the density of points falls off exponentially with increase in dimension making the separation problem correspondingly harder for algorithms to perform, and thereby providing an underestimate of the predictive strength of the optimal separation [20,21]).

The value of these SVM results of Fig. 4 is that they not only suggest the number of parameters that might need to be modified to relieve pathology (6–10), but also identify the individual parameters in order of importance. These parameters at the molecular level are therefore governing, in combination, the ability to define a plane or other surface that best separates the subspace of pathology from the subspace of normal physiology. We would then predict that a mathematical 'therapy' for our dystonic simulations could be effected by following the direction normal to this separation across that set of parameters. Going from mathematical therapy to patient therapy, these parameter changes would be brought about by using drugs that modulate that particular channel or signaling interaction.

In making the translation from simulation to therapy, we would want to remain mindful that some combinations of parameter alterations may tend to simply shut down the network, suggesting that the corresponding drug treatments might not be tolerated due to these types of side effects that are typically seen with drugs that reduce activity (e.g. benzodiazepines). For example, we can propose a hypothetical 4-drug cocktail using Fig. 4. We would start by addressing the first 2 parameters identified, the fast sodium and delayed rectifier potassium channels. These are of course the channels responsible for fast spiking. We might therefore start with a drug that reduces fast spiking, such as diphenylhydantoin. Noting now that the voltage-gated calcium channel parameter at the top of Fig. 4b is red in the third column, we might then consider the addition of a VGCC blocker, for example verapamil. Similarly, we would look for a drug that would augment K_A and a drug that blocks the ryanodine (RYR) receptor. We note that the mapping from parameter to drug will never be one-to-one. Most drugs have effects at multiple targets – so-called 'dirty drugs'. However, this is something that we could test directly in the model by modulating the multiple drug targets simultaneously, thereby making this limitation into a positive feature by identifying drugs with a particular molecular spectrum of action that more closely match directions in parameter space that are identified by the model.

Conclusions

Multiscale mechanistic simulations could be used to develop polypharmaceutical drug cocktails or to inform the use of multi-target therapeutic agents (dirty drugs) through parameter space assessments after separation of pathological from physiological activity patterns for dynamic diseases such as dystonia and epilepsy.

Conflict of interest

The authors have no conflict of interest to declare.

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Computational Models of Neurological Disorder

Modeling cytokine regulatory network dynamics driving neuroinflammation in central nervous system disorders

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A central goal of pharmacological efforts to treat central nervous system (CNS) diseases is to develop systemic therapeutics. This requires a fundamental understanding of CNS function within the organismal context. The immune system constitutes a key link between the periphery and CNS, and many neurological disorders and neurodegenerative diseases are characterized by immune dysfunction. We review the salient opportunities for applying computational models to CNS disease research, and summarize relevant approaches from previous studies. While the accurate prediction of disease-related phenomena is often considered the central goal of modeling efforts, we highlight the utility of computational modeling applications beyond making predictions, particularly for drawing counterintuitive insights from model-based analysis of multi-parametric and time series data sets.

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Introduction

Neurological disorders and neurodegenerative diseases of the central nervous system (CNS) typically exhibit slow progression to chronic pathology mediated by a multifactorial repertoire of elements. CNS diseases involve maladaptive neural circuits (e.g., Alzheimer's disease, AD), neuromodulation (e.g., Parkinson's disease, PD), and/or neurodegeneration (e.g., multiple sclerosis, MS). These diseases are multigenic, non-cell autonomous, and co-morbid with a number of organismal maladies including heart disease, metabolic dysregulation, and impaired immune function (Fig. 1a). Importantly, dysfunctional autonomic nervous system activity has been implicated in the pathology associated with neurodegenerative disease [1]. Numerous studies have established a role for neuroinflammation in CNS diseases. At the tissue level, neuroinflammation involves autocrine and paracrine cytokine signaling and interactions amongst vascular cells, infiltrating leukocytes, microglia, astrocytes, and neurons (Fig. 1b). Aberrant cytokine/chemokine regulation, endoplasmic reticulum stress, and mitochondrial dysfunction drive the upregulation of reactive oxygen/nitrogen species that contribute to neuroinflammation (Fig. 1c). The complexity of the multiscale regulatory networks and spatiotemporally distributed factors underlying CNS diseases precludes a direct

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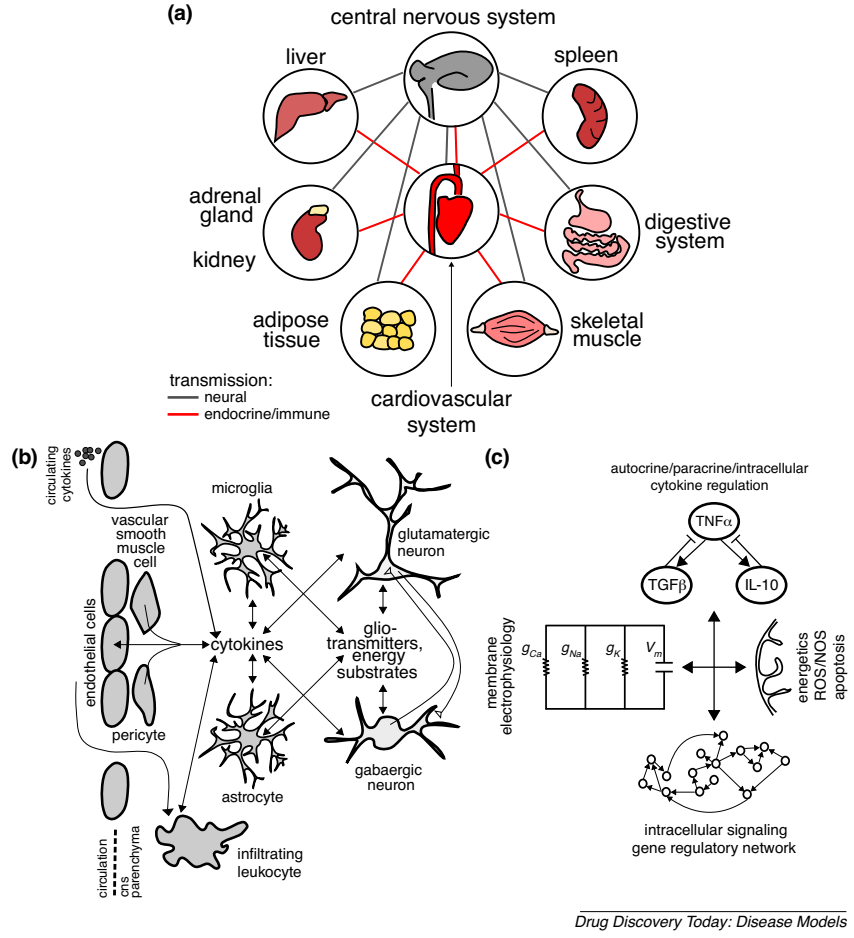

Figure 1. Distributed control in multiscale networks coordinating CNS function. **(a)** CNS function is coupled to the function of multiple organs through efferents/afferents and endocrine/immune transmission through the blood. **(b)** Tissue scale interactions amongst multiple cell types in the CNS. **(c)** Molecular scale networks regulating the integration of molecular signaling with neuronal physiology.

application of intuition to uncover the underlying principles of disease pathophysiology and the indentification of key control points for effective intervention.

Recent technological advances have led us into an 'omics' era in which it is reasonably cost effective and almost routine to obtain genomic, transcriptomic, proteomic, and metabolomic scale data, even from single cells. Analysis of such large-scale data has shown that mammalian tissue and cell type organization is far more complex than previously appreciated, and at the same time has begun to provide insights into the molecular and functional states of cells *in vivo*. For example, single cell transcriptomic data analysis uncovered 47 distinguishable neuronal phenotypes in the mouse cortex [2]. Similarly, single cell proteomic analysis has led to new insights in the cellular hierarchy and lineage progression of immune cells [3]. A quantitative understanding of how these multifactorial and multiscale components interact in regulatory networks is still elusive. Following others, we argue that computational modeling is necessary to make the next leap towards a comprehensive understanding of mechanisms driving neurological disorders and neurodegenerative diseases, and for developing effective therapeutic strategies [4,5].

Systems biologists have proposed to use modeling approaches to understand function in terms of principles that govern the interactions amongst biological elements, so as to facilitate a rational development of CNS disease therapeutics [6,7]. A hierarchy of control mechanisms regulate complex biological systems such as the CNS, ranging from molecular/cellular events to cell-cell interactions, along with the integration of signals from the blood and lymphatic systems [8]. Hence, a comprehensive understanding of the distributed multiscale control mechanisms of homeostasis – within the context of the organism – is central to understanding and tackling CNS pathogenesis [9] (Fig. 1a). Furthermore, peripheral inflammation is common in neurodegenerative

disease. It is likely the case that peripheral cytokines interact with central neuroinflammation through influences on the brain endothelium, and/or compromised blood–brain–barrier (BBB) (Fig. 1b). Hence, understanding the mechanisms driving peripheral inflammation is highly relevant to treating neurodegeneration.

In this review, we summarize the virtues and shortcomings of select computational modeling approaches, and highlight evidence that such approaches can be leveraged to define new biomarkers and therapeutics for CNS diseases. In particular, we review key literature related to the study of neuroinflammation in CNS disorders using computational modeling, and discuss the challenges and enabled opportunities.

Cytokine networks, glial phenotypes, and immune function

Proinflammatory cytokine upregulation and morphological adaptations of glial cells are the defining features of neuroinflammation. For instance, tumor necrosis factor-α (TNFα) upregulation was observed in AD, PD, and MS [10]. TNFα has been shown to yield excitotoxicity in neurons through effects on intrinsic membrane ion channels [11] (Fig. 1c). Furthermore, TNFα is known to induce apoptosis and necrosis, thereby exacerbating neurodegeneration [10]. Importantly, cytokines have been shown to interact through complex regulatory networks [12]. Thus, understanding the dynamics of cytokine interactions is critical to understanding the variegated and counterintuitive effects of glial cells on CNS phenotypes [13,14].

Microglia and astrocytes secrete and respond to an expansive repertoire of cytokines and chemokines. *In vitro*, cytokine networks can function through autocrine/paracrine signaling involving a single cell type. *In vivo*, cytokine network activity involves multiple cell types and cell-cell interactions, with prominent contributions from microglia and astrocytes (Fig. 1b). The effects of cytokines such as TNFα on cellular functional states have been considered in terms of feedback loops [12]. For instance, microglial activation mediated by TNFα upregulation results in the activation of multiple feedback cytokines that regulate the neuroinflammatory phenotype. The complicated crosstalk topologies of cytokine interaction networks highlight the necessity of computational approaches to disambiguate complex phenomena. It is well known that the expression level and activation timing of a cytokine determine its functional effects. However the influences of cytokine activation timing have not been thoroughly studied from an integrated network perspective *in vivo* in CNS disease and injury-related pathogenesis [15,16].

Cytokine network activity following inflammatory activation is associated with functionally relevant plasticity of glial morphology. Morphological plasticity can result in phagocytosis of boutons and spines, as well as physical displacement of synaptic terminals [17]. Inflammatory stimuli such as infection, trauma, or stroke elicit a phenotypic transition in microglia and astrocytes. Hence, both neurochemical and morphological aspects of glial activation in neuroinflammation cooperate to regulate neural network activity [18], however, the mechanistic coupling between cytokine network dynamics and morphological plasticity is only beginning to be revealed [19].

Computational modeling: conceptual motivation and implementation frameworks

Models abound in science. We all use them, though most of our models exist as implicit conceptual constructs and mental models rather than explicit formulations and mathematical representations. Understanding how CNS cellular phenotypes depend on the spatial location as well as amplitude and dynamics of molecular signals in the local microenvironment is critical for rationally developing therapeutic interventions against CNS disorders. Typical modeling studies entail modification or construction of a computational model, estimation of unknown parameters based on available data, testing the model predictions using data that were not utilized for parameter estimation, and simulation based analysis of the model. We argue that modeling is important for purposes beyond generating and testing predictions, including the following: to test/generate hypotheses, identify new questions, illuminate uncertainties, explore intervention strategies, explain complex phenomena, and achieve an integrated understanding of biological processes [20]. The knowledge derived from such approaches can yield important insights for drug development, such as whether a given property of a system has a physiological impact, is a consequence of another mechanism, or is an epiphenomena.

Models of inflammatory regulation include static and dynamic representations. Parameter-free simulations of network models can be implemented using Boolean logic, in which elements of the system are considered to be either 'on' or 'off' at a given time. While Boolean models provide a convenient framework for simulating the steady-state behaviors of networks inferred from high-throughput data, without the necessity for estimating uncertain parameters, this approach lacks biological plausibility insofar as the on-off digital representation of the molecular variables does not capture the dynamic and graded variations that are observed in complex biological processes. Dynamic modeling formalisms can be deterministic or stochastic. Network structure identification can be accomplished through measurements of responses to perturbations or inferences of molecular interaction coefficients that encode dynamics of the respective network [21,22]. Deterministic models are described by systems of ordinary or partial differential equations (ODEs, PDEs). Stochastic models incorporate biological randomness inherent to systems that involve molecular fluctuations. Deterministic models are relatively simple and efficient to

implement, with a limited degree of analytical tractability, and can be amenable to formal mathematical analyses that identify fundamental properties of the dynamic systems. Concepts from Boolean modeling and dynamic modeling can be integrated in multi-scale approaches. Agent based models (ABMs) incorporate interactions between cells that are characterized by specific states (e.g., activated, infected, or proliferating [23,24]).

Overview of cell signaling and immune modeling studies

We highlight select examples with relevance to studying cytokine networks in neuroinflammation. As an example of a statistical approach [25], investigators examined cytokine influences on apoptosis. The key feature of this study was the use of multi-perturbation, multi-parametric, time series experimental design that yielded a compendium of data. Janes et al. [25] analyzed this compendium using statistical modeling to identify novel molecular mechanisms connecting autocrine feedback loops involving IL-1α and TGFα signaling to apoptotic responses. Subsequent studies built on these data-driven modeling approaches to develop constrained fuzzy logic modeling of cell signaling [26] and gene regulatory networks [27]. These empirical, data-driven methods hold promise for expanding our knowledge beyond canonical cytokine signaling networks in order to fully interpret the dynamic patterns of cytokines and their molecular targets underlying neuroimmune processes.

In the context of deterministic and stochastic modeling frameworks, investigators have examined immune function and inflammation over a wide range of spatiotemporal scales. An illustrative example is the computational modeling of the receptor mediated activation of transcription factor NF-κB that transduces signaling responses to extracellular pathogens and cytokines, and regulates immune functions and apoptosis. Computational modeling of NF-κB activation revealed new insights into how different feedback regulators control distinct dynamic aspects of NF-κB level, localization, and activity [28,29,30]. An example of modeling at the tissue scale is the study of granulomas and associated immune response dynamics using a multiscale framework combining ODEs and ABMs. Cilfone et al. [24] modeled tuberculosis infection by simulating macrophages and T cells driven by intracellular cytokine signaling mediated by TNFα and IL-10 receptor activation. An important finding of this study was that an optimal balance of these cytokines was associated with minimized bacterial load. Simulation environments such as the Simmune tool provide systematic frameworks for representing molecular regulatory networks and cellular state transitions, and allow relating specific biochemical states to cellular scale responses such as division, death, migration, and secretion of cytokines and other factors [23]. Computational modeling studies such as the above provide key insights into the molecular regulation of emergent properties of biological systems from cells to tissues. Such model-driven insights can provide new hypotheses and predictions as well as motivate the need for reformulating the conceptual understanding of a biological phenomenon.

Computational modeling applications to study neuroinflammation and neurodegeneration

Statistical and simulation based modeling approaches have been undertaken to study CNS diseases. Zhang et al. [31] applied Bayesian network inference to identify molecular correlation modules from gene expression analyses of human brain samples from AD patients. This approach led to the identification of *TYROBP* as a regulator of microglia-mediated neuroinflammation in the prefrontal cortex. A temporal logic approach, similar to boolean logic, was implemented in simulations designed to examine how/why amyloid beta-stimulated microglia exhibit pro- and anti-inflammatory cytokine profiles in parallel, and how/why aged microglia fail to phagocytose elevated amyloid [32]. This work provided candidate explanations for key phenomena associated with AD. ODE-based studies of microglial cell signaling revealed that heat shock proteins may protect against stroke through inhibition of NF-κB signaling [33]. In the context of amyotrophic lateral sclerosis (ALS), modeling applications include statistical modeling of clinical data [34], tissue-scale modeling of cytokine regulatory dynamics [35], and single cell modeling of neuronal electrophysiology integrated with cellular energetics [36]. These studies elaborated our understanding of the multiscale mechanisms underlying ALS, suggested novel treatment regimens based on perturbing cytokine regulation, and identified clinical criteria for better prediction of ALS progression.

We have integrated computational modeling with experimentation in cellular, tissue-level, and organismal-scale studies of cytokine networks. At the cellular level, we modeled a microglia-specific autocrine/paracrine cytokine interaction network [37]. Surprisingly, we found that negative feedback inhibitors of TNFα showed divergent influences that were related to their relative dynamics. While TGFβ exhibited slower kinetics and facilitated the adaptation of TNFα to a sustained inflammatory stimulus, relatively fast IL-10 mediated feedback was associated with a counterintuitive decrease in adaptation. This finding was observed in experiments examining the LPS-induced cytokine response of bone marrow derived macrophages *in vitro* [37]. We developed a tissue-scale model of neuroinflammation including microglial and astrocytic contributions, and our simulations and experiments showed that IL-10 reduced TNFα adaptation *in vivo* [19]. Furthermore, we analyzed single cell multivariate microglial morphology data and found that morphological properties related to the shapes of somata and processes showed IL-10-dependent adaptation patterns. These model-driven studies demonstrated a novel link between cytokine network

dynamics and morphological features of microglia. We developed organismal scale models to simulate the development of dysregulated homeostasis in the context of autonomic nervous system dysfunction [22]. We applied systems identification techniques to infer dynamic multi-tissue gene regulatory networks involving cytokine transcripts in health and disease. Our analyses revealed that autonomic disease development was associated with a re-wired network structure and divergent activity patterns. We identified key regulatory elements with disease-specific molecular interactions and dynamic profiles, thereby providing candidates for further evaluation of compensatory responses to disease conditions, biomarker potential, and therapeutic interventions.

General principles of system function obtained from computational modeling and analysis

Design and control principles of CNS function elude simple intuition, necessitating an integrative and quantitative perspective. Computational modeling has highlighted the mutual influences of molecular kinetics and network structures in the context of process control mechanisms (e.g., adaptation and tolerance) and information processing (e.g., encoding and decoding). Multiple topologically similar but kinetically distinct feedback loops can exhibit differentiated functions to allow fine-tuning of system responses [37–40]. Through expansive searches of network structures, specific topological motifs have been implicated in emergent properties including adaptation to a sustained stimulus and priming/tolerance to repeated stimuli [41,42], both of which are critical to immune function and have been studied in cytokine networks [43,37]. However, as a note of caution, some of these findings may be dependent on the mathematical framework utilized (e.g., Hill equations to describe interaction rates) and do not necessarily generalize for applications to identical topological motifs with different mathematical formulations of the dynamics [44]. The encoding and decoding properties of a given signal are governed by dynamic characteristics including delay, onset duration, amplitude, signal duration, deactivation, and frequency [45,46]. Stimulus-specific feedback interactions have been shown to impart cytokine stimulus-specific coding properties as reflected by distinct dynamic and transcriptional responses [47,48]. Individual features of signaling dynamics can be insufficient for determining system response profiles [46], thereby providing a quantitative explanation of why individual therapeutics, as opposed to combination therapies, are often insufficient to revert diseases. Furthermore, these findings support the notion that dynamic properties of a signal can be considered as effective targets for therapeutic intervention [49]. The aforementioned examples highlight the complex mapping between stimuli and network responses that can be unraveled using computational modeling.

Opportunities for applications in CNS drug discovery

CNS disease research could greatly benefit from integrated computational and experimental approaches for identifying novel diagnostic/prognostic biomarkers, drug targets, dosing regimens, adverse drug responses, and patient-specificity. Here we outline a few opportunities to facilitate the use of computational approaches based on acquiring necessary data, taking advantage of approaches utilized in other fields, and reformulating the conceptualization of disease initiation, progression, and response to intervention. Overall, the applications suggested to advance drug discovery for neuroinflammation and neurodegeneration include addressing a set of questions aimed at identifying key elements, interactions, and dynamics that serve as control points for effective intervention: What are the critical molecular mechanisms that govern cytokine regulation within the local neural tissue microenvironment? How to integrate contributions of multiple cell types within the neural tissue across relevant timescales? How to couple functional responses of distinct neural and immune cell subpopulations to changes in neuronal physiology? What are the relative contributions of genotype and physiological phenotype in shaping response to neuroinflammation? How to account for the intrinsic and inter-individual variation in inflammatory responses and progression of neurodegenerative disease?

The time courses of disease progression, and variability thereof across human populations, are inadequately understood. Multiple temporal profiles could exist, with distinct implications for therapeutic approaches (Fig. 2a). In this regard, understanding the dynamics of the molecular and cellular networks may provide a more tractable way to differentiate disease risk and treatment viability than considering genetics alone [50]. In understanding the intrinsic variation, a critical unresolved issue is the question of cell type contributions to the inflammatory milieu in the CNS. In addition to microglia and astrocytes, other cells including neurons, endothelial cells, and pericytes are known to secrete cytokines. Similarly, vascular smooth muscle cells have been demonstrated to undergo phenotypic transitions into macrophage-like cells under inflammatory conditions [51]. However, the extent of cell phenotype plasticity and variability, and relative contributions of diverse cell types to tissue levels of cytokines, are unknown. These questions can be addressed through gene expression profiling of single cells in tissue sections using laser capture microdissection [52,53]. In addition, proteomic analysis optimized for single-cell scale samples would be very useful for determining the actual levels of the cytokines within the localized microenvironment. In general, this issue of cell type contributions to overall cytokine levels must be resolved to achieve a comprehensive understanding of cytokine network function and the mechanisms of neuroinflammation. This information is also critical to appropriately specify the structure and parameterize a

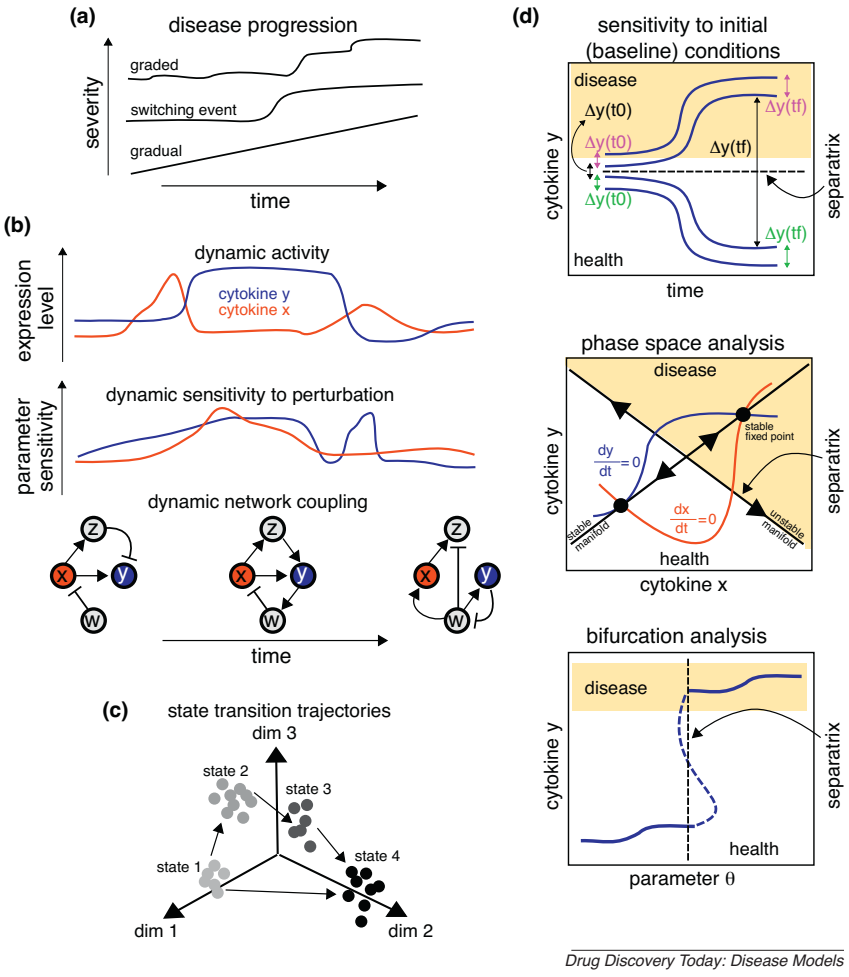

Figure 2. Modeling disease dynamics. **(a)** Potential dynamics of disease progression. **(b)** Illustrative examples of dynamics in molecular levels, parameter sensitivity, and molecular regulatory network structure. **(c)** Illustrative trajectories of state transitions derived from statistical analysis of high-dimensional data and visualized in a reduced dimensional space. **(d)** Graphical representations of mathematical analysis to identify thresholds of disease – or separatrices – based on changes in initial conditions, variations in levels of system elements (e.g., cytokines x and y), and variations in parameters (e.g., network interaction coefficients).

computational model of the multicellular network underlying neuroinflammation (Fig. 1b). Similarly, understanding the mechanisms underlying multi-organ communication could be informative to formulate and parameterize corresponding computational models to study how neuroinflammation is regulated in an organismal context (Fig. 1a).

Understanding the dynamic mechanisms of disease is critical for identification of new biomarkers and drug targets. Given an ODE model of a CNS disease, computational studies can help to elucidate the relationship between the temporal evolution of a system response and the sensitivities of the underlying elements to a therapeutic perturbation (Fig. 2b). Sensitivity analyses have shown that specific molecular elements are predominantly important for system behavior at particular times, whereas interventions at other times were ineffectual [54]. Importantly, these results suggest that successful precision medicine requires an understanding of when a specific molecular intervention should be applied for optimal effect. It is becoming increasingly clear that varying stages of a disease progression are associated with distinct molecular interactions or 'differential networks' (Fig. 2b; [55]). Hence, models of CNS disease progression should consider cellular and molecular networks that are defined by dynamic rather than static connectivity structures.

From a statistical perspective, global molecular configurations can be defined by projecting high dimensional multivariate data onto a lower dimensional subspace that can be analyzed for trajectories of state transitions associated with disease processes, as well as for mapping alternative trajectories that could account for inter-individual variability and therapeutic responses (Fig. 2c). Such approaches have provided important insights into both developmental processes and drug responses [3,56]. Network modeling based on protein-protein interactions has also facilitated understanding of

potential molecular underpinnings of seemingly unrelated diseases. Furthermore, the module of disease genes identified through network analysis was enriched for targets of drugs with adverse event profiles [57]. These data-driven methods could be integrated with dynamic modeling by distilling high-throughput data sets into sets of response profiles that are used to constrain network structures and fine tune model parameters, as well as experimentally characterize the landscape of state transitions.

Mathematical analyses of dynamic models facilitate a comprehensive analysis of the landscape of transitions between health and disease, to both identify and elucidate the mechanistic basis of transition control points, in time or molecular space, that demarcate qualitative shifts from health to disease (Fig. 2d; [58]). For instance, the initial levels of system components have been shown to regulate cytokine network dynamics and apoptotic responses to cytokine treatment ([59,37]; Fig. 2d, top). Similarly, related approaches involving phase space analysis (Fig. 2d, middle) and bifurcation analysis (Fig. 2d, bottom) can be pursued to understand the conditions underlying critical transitions to pathological states [60,61]. Such analyses further aid in identifying biomarkers that distinguish disease trajectories (as opposed to instantaneously observed states), for identifying early-warning signals of critical transitions driven by stochasticity, and for predicting effective targets that can prevent transitions to disease or reverse the course towards a healthy state. Such approaches can be utilized to enhance our understanding of the molecular trajectories through which cytokine dynamics promote robust neuroinflammation and CNS disease states.

A recent application of computational models for exploring disease dynamics is the simulation of a population of ''virtual patients''. The objective is to account for inter-individual variability by considering a large set of simulations based on population-relevant and physiologically meaningful ranges of parameters [62]. These approaches typically utilize statistical and clustering analyses to identify patterns in network dynamics and attempt to relate the patterns of disease dynamics to distinct underlying parameters, providing new biomarker and drug target candidates. These models can differentiate disease subtypes and the patient-specific drug responses [63]. Considering the intrinsic stochasticity and extent of uncertainty in the neuroinflammation network structures and parameters, such model-based large-scale exploration is crucial to evaluate the very many possibilities in which disease dynamics may unfold. The computational model-based simulation of virtual patient populations holds promise in advancing neuroinflammation and neurodegeneration research by taking an unbiased perspective.

Conclusions

While methodological advances, increased software availability, and enhanced computational speed facilitate the integration of high throughput experimental data acquisition, analysis, and computational modeling, paradigm shifts that transform understanding are driven by conceptual advances. Understanding pathophysiology is typically formulated as a problem of deconvolving cause from consequence of CNS disease. As stated, this task is particularly difficult in human diseases. We argue that the 'cause versus consequence' dichotomy is a flawed notion when applied to progressive disorders that involve multiple levels of a complex hierarchical network. Conventional experimental designs may be inadequate for generating understanding of CNS disease. For instance, targeted knockouts (KO) or overexpression experiments may not permit an unambiguous understanding of the functional role of the targeted molecule in disease due to adaptation and compensation. This difficulty is exemplified when there is dynamic sensitivity of the system response to perturbations. The advancement of experimental studies tracking the molecular, cellular, and physiological dynamics of disease progression through fine-grained time-series, and interpreting these data using multiscale modeling, can lead to new insights into neuroinflammatory process dynamics and control principles. Combining such an approach with typical manipulation experiments will help to elucidate the contributions of molecular and cellular elements to disease dynamics, significantly advancing the quest for novel intervention strategies and therapeutic targets in CNS diseases.

Conflict of interest

The authors have no conflict of interest to declare.

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Computational Models of Neurological Disorder

The use of dynamic computational models of neural circuitry to streamline new drug development

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We propose a relatively novel use of dynamic models of neural circuitry in the process of new drug development for neurological disorders. A neural circuit model of depressive disorder was developed. Differences in synaptic activation represented variations in drug binding affinity strength for ten putative molecules. Circuit dynamics led to changes in firing rates compared to normal and depressed baselines. Differing abilities to affect circuitry dynamics not linearly related to binding affinity were appreciated, allowing molecules to be prioritized earlier for future study. Animal and human trial planning can use this information to streamline drug development, conserving cost and time.

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A recent comprehensive study by DiMasi *et al.* [1] of the cost of developing 106 drugs found that out-of-pocket research and development (R&D) costs averaged $1.4 billion per drug, while including the cost of capital employed raises the average total to $2.5 billion, in line with other similar studies [1–3]. Despite the advent of new drug development tools, this study found costs had *increased* at an average rate of 8.5% per year above the rate of inflation [1].

Key to their methodology and understanding of the underlying reasons for high costs is tying the costs of *unsuccessful* drugs to the costs of the successful, eventually-approved drugs. The clinical success rate reported in their 2003 study was 21.5%, while the 2016 study found the rate had dropped to 11.8%, consistent with success rates reported in other studies [1,5]. Offsetting declining clinical success rates is the ability of companies to abandon drug candidates predicted to fail earlier in the process [1,4] and use computational models to provide early inexpensive predictions [5].

The average time from drug candidate synthesis to marketing approval is typically over 10 years [1]. In general, R&D costs rise throughout the development cycle and thus the earlier a company can eliminate candidates likely to fail, the greater the savings and lower the opportunity cost [1,6].

In silico neural circuitry testing in early drug development

Given the staggering, increasing expense of drug R&D, the R&D timeline, and the probability that animal models may fail to predict human trial results, we propose the

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development of *in silico* neural circuitry models as a highly cost-efficient adjunct for streamlining and prioritizing prospective molecules for treating neurological disorders, which impose abnormally high burdens on society [7], early in the process, before animal trials. Similar to other efforts [8–14], such a paradigm holds promise for minimizing loss of resources earlier in the process than heretofore possible and prioritizing molecules with higher probabilities of success. To the best of our knowledge, this is the first proposal of this kind.

Computational models of psychiatric and neurological disorders

Computational studies of psychiatric or neurological disorders, often taking a systems biology approach, have been performed for several decades [15–19]. Many are explicitly directed to inform pharmacotherapy [20–22] or address drug development with different tools than neural circuitry modeling [8,15,23]. Two notable exceptions are Ramirez *et al.* [19] and Siekmeier and vanMaanen [24], both published only in the preceding three years.

Siekmeier and vanMaanen used a network model of just 160 hippocampal pyramidal cells and 80 interneurons, yet each cell was detailed at the synaptic level (e.g. 40–60 dendritic compartments, Hodgkin–Huxley ion channel dynamics for 3 ions) to the degree that required a supercomputer to do parameter sweeps in their 'virtual medication trials' [24]. The purpose of their study was to find novel drug targets to treat schizophrenia, which they represented by changing conductance and weight of gamma aminobutyric acid (GABA) receptors, and they screened for efficacy by adjusting conductances of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and MK-0777 to represent drug effects and calculated power spectra of the 'schizophrenic' neural circuit under a sample task as compared to healthy baseline.

Ramirez *et al.*'s network model of major depressive disorder (MDD), incorporated computationally far simpler spiking neuron and firing rate neuron models than Siekmeier and vanMaanen's to explain the etiology and treatment of MDD via SSRIs (selective serotonin re-uptake inhibitors) and deep brain stimulation [19].

Recently there have been significant attempts to create a 'systems pharmacology' approach, stemming from the systems biology philosophy but specifically addressing drug development and how to capture the correct level of detail at a higher systems level than previous systems biology approaches, to predict the results of pharmacological intervention *in vitro* and *in vivo* [5,25]. Noting the high failure rate of central nervous system (CNS) drugs, Geerts *et al.* have developed a 'quantitative systems pharmacology' (QSP) paradigm for drugs targeting CNS [26–28].

A key challenge to computational psychiatric or neurological disease modeling is to efficiently bridge the explanatory gap from changes at the molecular level to associated changes at the cellular, tissue, network, and in the end, clinical, that is, behavioral, level [15–17,21,22,25,29]. Wang *et al.* note that computational studies of mechanism of action at the synaptic level offer the potential to be rigorously calibrated quantitatively, such as when incorporating binding affinity data [21].

Spiros *et al.* envision that, in their QSP paradigm, different approaches may be taken to tie changes at the neurotransmitter-receptor level to CNS drug target clinical outcomes and as an example, use information capacity of thalamic spike trains within the cortical–striatal–thalamo–cortical loop to correlate drug candidates with schizophrenic outcomes measured by PANSS (positive and negative syndrome scale) [30].

We present here a different example of how these new approaches to early drug testing *in silico* might work, using a relatively sophisticated biologically-inspired dynamic model of neural circuitry known to be relevant to MDD and the resulting effects of 10 different molecules whose only available information are taken to be binding affinities for a particular receptor known to exist in a few areas within that circuitry.

Neural circuit model of major depressive disorder. Toward the goal of modeling drug effects on MDD we sought to adapt our existing neural circuitry simulator, capturing large-scale circuit effects, to represent aspects of these putative molecule-receptor actions on small groups of neurons within the MDD neural circuitry. Alterations in overall firing rates within each nucleus or group of neurons could then be evaluated as if the new molecule had been used in the organism itself.

Simulation methods and depression model

The neural circuitry simulator, UNCuS (Universal Neural Circuitry Simulator), is described in Arle *et al.* [31–33]. UNCuS was designed to use biologically based cell membrane parameters, dendritic tree processing, and synaptic representations within individually-rendered neuron models connected via axons of specified axonal delays in arbitrary configurations. Large-scale circuit-level dynamics representing known anatomy and physiology can be created that include hundreds of thousands or more neurons and millions of synapses using time steps of 0.25 ms. We developed a model of MDD based on a variety of circuit-analyzing techniques summarized in a review of brain reward circuitry [34]. Our MDD model used here included 2805 neurons and 29,411 synapses for this proof of concept study.

A single action potential occurs over the course of 1 ms. The simulation time step was 0.1 ms. Thirteen parameters in UNCuS allow replication of desired neuron characteristics and a jitter of white noise is added to each neuron's parameters in a default range of 10%. Additional parameters include the polarity (excitatory or inhibitory), connectivity

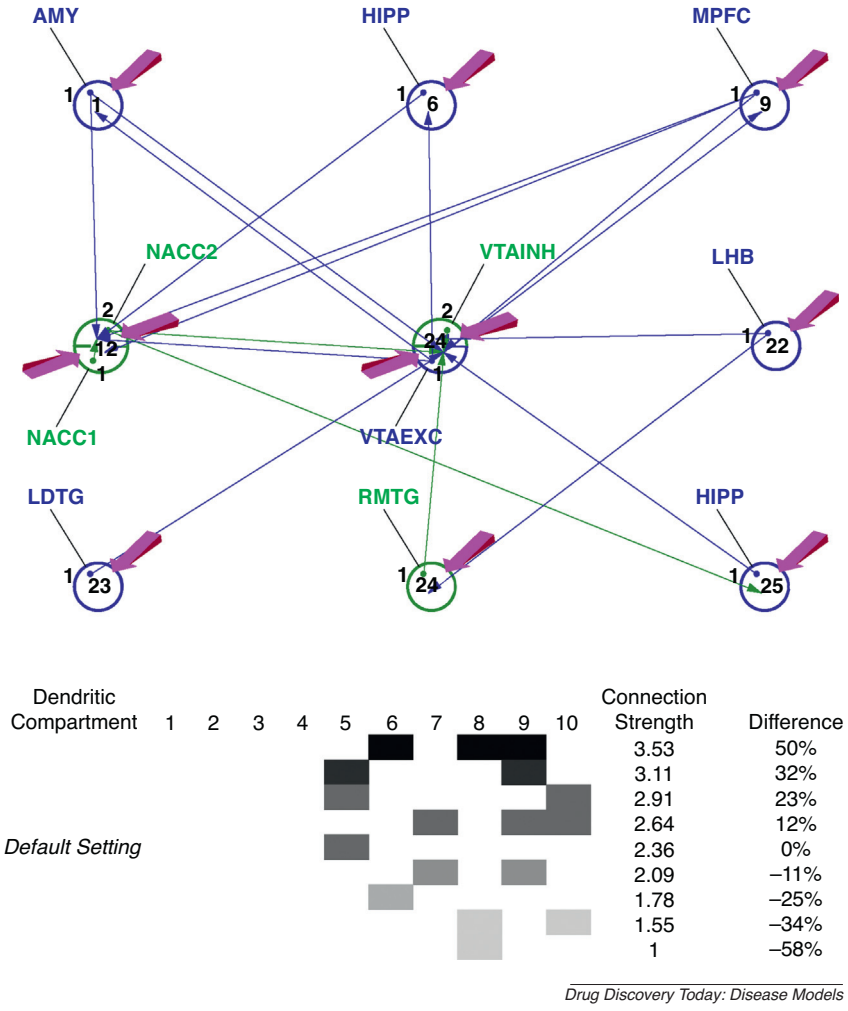

Figure 1. Universal neural circuit simulator (UNCuS). Top: Screenshot of neural groups and their connectivity in UNCuS. Blue: excitatory groups and connections. Green: inhibitory groups and connections. Magenta arrows: random intra-cellular stimulation applied to each group to set its reported background firing rate. The numbers uniquely specify each neural group by its 'population' (external number and circle) and 'group' (internal number for each division in the population). Bottom: Diagram of UNCuS neural dendritic compartment combinations used in this study to simulate effects of putative drug binding affinity on connection strength between neural groups. Ten compartments, each set to 'on' or 'off', provide 2^{10} or 1024 possible strength settings with a 32-fold dyamic range. The default setting for all connections before modifying them to simulate depressive disorder was compartment 5 on, all others off. These patterns of synaptic strength change were only applied at the few target pathways where the putative 'drug molecule' would act.

(topographic or random), connection strength in density or contacted dendritic compartments (Fig. 1 bottom shows 10 compartments, which can be in 'on' or 'off' states, allowing a total of 2^{10} or 1024 strength variations), and axonal delay between each neural group and its target, stimuli (e.g. random or electrode), number of neurons in the group, and others.

For this study, the principal parameters used were topographical connectivity between neural groups, the connection strength and polarity between them, their background firing rate as found in the literature and modulation of the firing rate from that baseline rate to simulate the abnormal levels seen in depression or the non-depressed state. Background rates were set by adjusting the random stimulation function on each neural group in UNCuS (Table 1). A standard neuron with a linear current–voltage response curve was used for all groups, and the initial firing activity of each group was tonic (i.e. steady-state), while circuit dynamics determined subsequent patterns. Overall, 11 nuclear or sub-nuclear cell groups of neurons were created.

Major depressive disorder neural groups. The key neural groups and connections between them in our model are shown in Fig. 1, top. Several variations of the model were built, starting with a Healthy Control (HC) model. In the HC model all connection strengths were set with dendritic compartment 5 activated (the middle of the dendritic tree electrotonic equivalence cylinder) and the other 9 compartments off (Fig. 1, bottom). As is the case *in vivo* [44], the connection strength conveyed by each compartment is highest closest to the neuron (i.e. lower compartment numbers and to the left in Fig. 1, bottom).

Table 1. Neural groups in the brain reward circuitry included in the model. Their polarity (excitatory and inhibitory) relative to their target group are given, along with the neurotransmitter involved, the target firing rate drawn from the literature, and the actual baseline rate in the Healthy Control neural circuitry model. Note that two cell types and groups are included for the nucleus accumbens and ventral tegmental area. GABA: gamma aminobutyric acid.

Neural group	Polarity	Neurotransmitter	Target baseline Firing rate (Hz)	Calibrated HC Firing rate (Hz)	Source
Amygdala	Excitatory	Glutamine	3	3.9	[35]
Hippocampus	Excitatory	Glutamine	<1–10	2.8	[36]
Lateral-Dorsal-Tegmentum	Excitatory	Glutamine	8.24 ± 3.37	10.1	[37]
Lateral-Habenula	Excitatory	Glutamine	6.7 + −0.8	6.7	[38]
Lateral-Hypothalamus	Excitatory	Glutamine	~3	3.1	[39]
Medial prefrontal cortex	Excitatory	Glutamine	5–10	7.9	[40]
Nucleus-accumbens-1	Inhibitory	GABA	7	7.0	[41]
Nucleus-accumbens-2	Inhibitory	GABA	7	7.2	[41]
Rostromedial-Tegmentum	Inhibitory	GABA	18	17.5	[42]
Ventral-tegmental-area	Excitatory	Dopamine	1.2	1.2	[34,42]
Ventral-tegmental-area	Inhibitory	GABA	~18	17.5	[43]

Major depressive disorder calibration. We simulated two aberrant circuit conditions of depression:

(1) increased firing in the ventral tegmental excitatory group (VTAe), affecting in particular the nucleus accumbens (NAc) [34], via increasing its firing rate by 67% as reported in a mouse model of depression [45]
(2) reduced glutamatergic expression between the medial prefrontal cortex (mPFC) and NAc [34] by reducing its connection strength to the two NAc inhibitory groups via activating dendritic compartments 8 and 10 instead of 5 (a reduction of ~58% on a scale with a dynamic range of ~3200% between all compartments on and only one on; see Fig. 1).

We then combined these two conditions in the baseline depression model before simulating drug action on the circuit. The effects of each condition on firing rates, and the combined effect, as compared with the Healthy Control model, are shown in Table 2. Firing rates were averaged across the first 100 cells in each neural group over 500 ms runs in 0.25 ms time steps.

Simulated drug action was achieved by changing the connection strength between certain source groups and a subset of their targets (via the dendritic compartment table, Fig. 1, bottom) as would be effected by the varying binding affinity of different drugs targeting the same neural group, for example, receptor agonists or antagonists at any site of action [46,47], similar to Siekmeier and vanMaanen [24], assuming a steady-state concentration profile.

Drug simulation results

Experiment 1. The first drug action simulated was motivated by aberrant circuit condition (1) above, to attempt to reduce the effect of excessive VTAe on its NAc target by modulating the connection strength of the inhibitory GABAergic rostrotegmedial tegmentum (RMTg) connection to VTAe. The results of 8 variations in connection strength are shown in Fig. 2, top. Note in particular the reduction of excessive firing in the VTA via decreasing inhibitory connection strength from RMTg; it is reported that reducing the firing rate short of healthy baseline in a mouse model of depression with lithium treatment was sufficient to ameliorate mania and anxiety symptoms, but not those of depression symptoms [45]. It could be that further reduction of VTAe firing rates is required to ameliorate depression, or another mechanism is at work and firing rate is epiphenomal, although the authors state that previous studies indicate this is unlikely [45].

Summary of significant simulated drug effects. Predictably, as noted, the greatest reduction in VTAe firing rate correlated with the greatest increase of its connection strength of the inhibitory source group RMTg. Less predictably, however, were other notable epiphenomenal effects of optimizing this connection by changing connection strength between the groups, comparing firing rates with the Healthy Control baseline rates, and considering the rate of the group targeted by the drug that is closest to its baseline rate as optimal. Using the same metric, some collateral effects on non-targeted groups appear to be beneficial and some to be detrimental (Table S1 online supplement; compare the approaches taken in Ramirez *et al.* [19] and Spiros *et al.* [30]).

Experiment 2. We simulated a second drug action to attempt to correct aberrant circuit condition (1) by increasing the excitatory connection strength from the lateral habenula (LHb) to the ventral tegmental area GABAergic *inhibitory* group (VTAi), an interneuron group that inhibits VTAe, which in turn, as noted, targets NAc via a dopaminergic connection. The results of 8 variations in connection strength are shown in Fig. 2. This effort was not as effective in reducing the firing rate of VTAe as in experiment 1, again falling short of returning VTAe to its Healthy Control

Table 2. Comparison of firing rates in neural circuitry model of depression. Shown are the neural groups included in the model their average firing rates (Hz) averaged over 500 ms, and the percentage change in firing rate between the Healthy Control (HC) and depression models. Two depression conditions were simulated separately and then combined. Depression 1 (D1): increased firing in the ventral tegmental excitatory group, affecting in particular the nucleus accumbens (NAc). Depression 2 (D2): 58% reduction of glutamatergic expression between the medial prefrontal cortex (mPFC) and NAc by reducing mPFC connection strength to the two NAc inhibitory groups via activating dendritic compartments 8 and 10 instead of 5 (see Fig. 1).

Neural group	HC	D1	D1/HC	D2	D2/HC	D1 + D2	D1 + D2/HC
Amygdala	3.9	4.3	10.3%	4.2	7.7%	4.4	12.8%
Hippocampus	2.8	3.2	14.3%	3.2	14.3%	3.5	25.0%
Lateral-dorsal-tegmentum	10.1	10.1	0.0%	10.1	0.0%	10.1	0.0%
Lateral-habenula	6.7	6.7	0.0%	6.7	0.0%	6.7	0.0%
Lateral-hypothalamus	3.1	3.1	0.0%	3.7	19.4%	3.4	9.7%
Medial-PFC	7.9	8.4	6.3%	8.3	5.1%	8.7	10.1%
Nucleus-accumbens 1	7.0	7	0.0%	4.1	−41.4%	4.1	−41.4%
Nucleus-accumbens 2	7.2	7.9	9.7%	6	−16.7%	6.7	−6.9%
Rostromedial-tegmentum	17.5	17.5	0.0%	17.5	0.0%	17.5	0.0%
Ventral-tegmental-area-exc	1.2	2	66.7%	1.8	50.0%	2.7	125.0%
Ventral-tegmental-area-inh	17.5	17.5	0.0%	17.5	0.0%	17.5	0.0%

baseline. (Significant effects of Experiment 2 are noted in Table S1, online supplement.)

A place for neural circuit simulation in neurological drug development

Drug development in neurological disorders must not only rely on solving the complexities of finding higher or predictable binding affinities for individual targets within the appropriate neural circuitry [48–53], but also handle the less predictable *dynamics* of their effects *on* the neural circuitry itself. Such changes may be non-linear, unpredictable and emergent [15,29], and add significantly to the increasing costs of research and development. We present here a proof of concept study wherein neural circuitry simulations of a

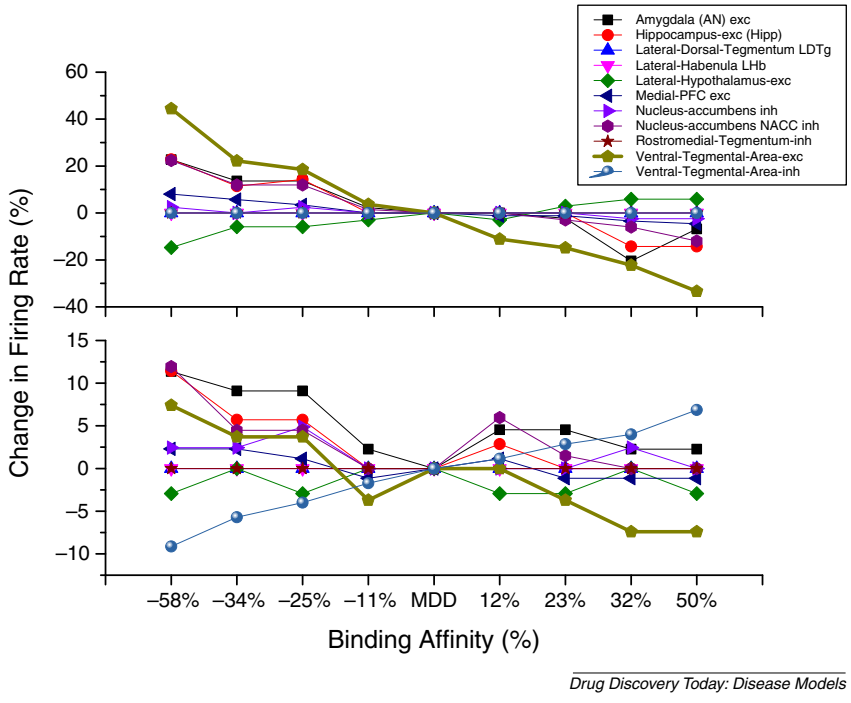

Figure 2. Simulation of putative drug action on neural circuitry model of depression. Top panel: modulation of inhibitory connection strength of GABAergic RMTg group on VTAe excitatory dopaminergic group. Shown are the firing rate of each neural group in the model versus their baseline in the MDD model for decreases and increases in connection strength between RMTg and VTAe as a drug targeting the GABAergic neurotransmitter-receptor system at the synapses of the VTAe might affect them. Bottom panel: modulation of excitatory connection strength of glutamatergic LHb on GABAergic VTAi, which in turn inhibits VTAe, as a drug targeting the glutamatergic neurotransmitter-receptor system at the synapses of the VTAi might affect them. *Abbreviations*: GABA: gamma aminobutyric acid; MDD: major depressive disorder; RMTg: rostromedial tegmentum; VTAe: excitatory ventral tegmental area; VTAi: inhibitory ventral tegmental area; LHb: lateral habenulum.

neurological condition (MDD in this case) are used to examine the effects of a series of putative molecules in early drug development on circuitry dynamics in order to streamline and prioritize prospective molecules for further research, such as animal studies.

The UNCuS depression model was based on the data and model of abnormalities in the brain reward circuit [34]. A group of 10 putative drug molecules with different binding affinities for a receptor located in a select group of neurons within the circuit were examined. The resulting firing rate dynamics suggest that while some activity in the circuit, such as in groups monosynaptically connected to the drug-affected group, were predictably related to binding affinity, many other activity changes in other neural groups were not predictable. As shown in Fig. 2, while the firing rate of monosynaptically targeted groups is often monotonic in response to strengthening or weakening binding affinity, the actual rate, which can have significant effects on behavior [15], can only be determined by calibrating the background firing rates and connections based on the literature and running the model. This is the critical finding and strongly suggests that cost and time in development can be reduced if those less-predictable effects could be simulated early in the overall process.

In experiment 1, for instance, firing rates generally change monotonically, but particularly with reductions of binding affinity, we see non-monotonicity in firing rate changes of some groups (e.g. amygdala, hippocampus). In experiment 2, greater unpredictability is shown, for example, in nucleus accumbens, hypothalamus, and amygdala.

The model presented here for proof of concept is simplistic, as are many in the literature, for a variety of reasons (see, e.g. the limitations given in Ostaszewski *et al.* in particular of network construction [15] and Ramirez *et al.* [19]). Increasing the number of connections and, notably, recurrent connections in the circuitry, would produce even further unobvious and unpredictable effects.

Limitations. Several areas of limitations are obvious in this proof of concept study. Most overtly, we cannot hope to simulate every anatomical and physiological component of the neural system being examined. Not all connections to and from groups have been included. This is typically the case since a model of the entire brain has not been constructed, thus one tries to incorporate only what is necessary and sufficient to reproduce phenomena of interest and calibrate to what *has* been shown in the literature [19]. Likewise, not all groups in each network were included, only those specified in Russo and Nestler [34]. One method we used to account for these omissions was to calibrate the firing rate (active or resting state) of each group as reported in the literature, thus attempting to include the net effect of all groups, included and not included, in the model. In this approach a further limitation is that some reported firing rates are from animals rather than humans.

Last, translating values for binding affinity can be difficult and may be inaccurate in terms of each molecule's true post-synaptic effects. However, as information becomes available about the actual post-synaptic receptor effects from each molecule, the simulations can be refined and made more accurate.

Conclusion

Neural circuitry simulations tied to known or suspected drug effects at one or more of the dozen or so sites of action [47] could be used to see what overall circuit effects result from various drug parameters.

Because the dynamics of complex neural circuitry make predictions of the effects of a particular drug prospect less than obvious, the ability to use such models to give an early hint at the overall resulting dynamics with any given molecule provides a way to prioritize molecules for study in animal trials more efficiently. Hundreds of putative molecules could be reduced to perhaps a 'top 10' – those seen as more likely than the others to yield neural circuitry dynamics most consistent with known clinical success. Helping to increase 'hit' probabilities at such an early stage in drug development could theoretically save millions of dollars per drug in R&D costs alone.

Authorship statement

JEA and KWC developed the methods, KWC implemented the model. Both authors reviewed and approved the manuscript.

Conflict of interest statement

The authors have no conflict of Interest related to the work in this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ddmod.2017.01.002.

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Computational Models of Neurological Disorder

Computer modeling of ischemic stroke

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The occlusion of a blood vessel in the brain causes an ischemic stroke. Current treatment relies on restoration of blood flow within 3 hours. Substantial research has focused on neuroprotection to spare compromised neural tissue and extend the treatment time window. Despite success with animal models and extensive associated clinical testing, there are still no therapies of this kind. Ischemic stroke is fundamentally a multiscale phenomenon where a cascade of changes triggered by loss of blood flow involve processes at spatial scales from molecular to centimeters with damage occurring in milliseconds to days and recovery into years. Multiscale computational modeling is a technique to assist understanding of the many agents involved in these multitudinous interacting pathways to provide clues for *in silico* development of multi-target polypharmacy drug cocktails.

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Introduction

Stroke is a major cause of death or disability, the 5th leading cause of death in the US with prevalence of ~2.6% of the adult population [1]. Around 85% of strokes are ischemic [2]. Neuroprotective strategies would target the myriad destructive processes that occur following a stroke, including spreading depression, excitotoxicity, cytotoxic edema, accumulation of free radicals, etc., in order to extend the treatment time window and reduce the volume of damaged brain tissue. Here we review current computational modeling efforts and consider their usefulness as a bridge for drug discovery which will help close the current gap between animal models and clinical trials.

Background

The many causes and types of stroke require vastly different approaches to prevention and treatment, and vastly different approaches to the type of multiscale modeling (computer simulation) that is used. For example, prevention of hemorrhagic stroke due to a blood vessel aneurysm must be understood in terms of laminar or turbulent blood flow under pressure and the various layers of the blood vessel that must fail, to give rise to this type of stroke; treatment would then be surgical prophylaxis for aneurysms deemed prone to bursting. Modeling ischemic stroke could also focus on the vascular system but would include pathophysiology of platelets, clotting cascades in the blood, to determine the likelihood of thrombosis formation. Such a kind of modeling approaches are particularly valuable to further understanding of the current standard of care through reperfusion. By contrast, cardiogenic stroke syndromes and preventive therapy would best be understood by modeling the left atrium of the heart. In this review, we will not look at either of these stroke types but will instead focus on the effects of ischemia on brain tissue. Of course, even the brain sans blood and blood vessels

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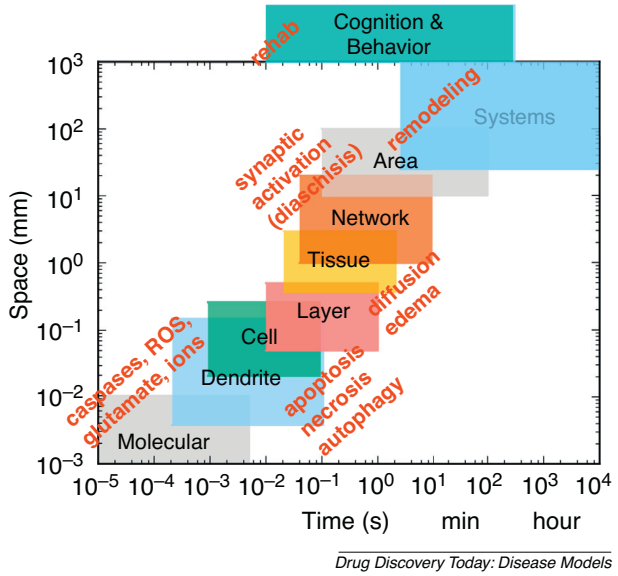

Figure 1. Many scales of brain organization inform our understanding of stroke with different responses to stroke ranging in time from milliseconds to years. In this review we focus on the changes that occur at molecular, cell, tissue and network levels, noting the interplay of neuronal network synaptic interactions with bulk tissue interactions.

is itself a complex subject with many scales of interactions from molecular to tissue and network levels (Fig. 1). Within the brain, study of stroke includes additional topics of brain response, relevant to neurorehabilitation and recovery, as we have discussed elsewhere [3,4].

Damage to tissue in ischemic stroke is divided into three regions: (1) an infarct core where cells suffer irreparable damage and death, (2) a penumbra where cells may recover with reperfusion or with the assistance of neuroprotective therapy, (3) a further region of edema (benign oligemia) where spontaneous recovery is expected (Fig. 2).

The penumbra, vulnerable but salvageable, is the target of *neuroprotection strategies* (Fig. 2c). Unfortunately, despite thousands of experimental studies on neuroprotection, and hundreds of clinical trials, there are no approved neuroprotective drugs [5]. Agents that have been tried include Ca^{2+} antagonists [6], NMDA receptor antagonists [7] and free radical scavengers [8]. Neuroprotective agents that achieve substantial tissue sparing in animal models have resulted in high profile clinical-trial failures. The *clinical translation* problems result both from discrepancies between the experimental and clinical treatments and from underlying biological differences between test animals and humans.

Multiscale mechanistic computer simulation has the potential to bridge the gap between animal models of ischemic stroke and patient outcomes and to facilitate development of drug cocktails by improving our understanding of the many agents and processes involved in cell death. Computer modeling will elucidate the many nonlinearities in time (early, mid, late death) and in space (multiple cores and multiple penumbra). The volume of penumbra decays exponentials as cells die and the core expands. It has been estimated that ~2 million neurons die every minute following a stroke [9]. However, the many causes of cell damage and death are produced by feedback loops which are highly nonlinear (Fig. 3), and this average value of 2 million is expected to be unevenly distributed in both time and space.

Multiscale computational modeling in neuroscience is based on biophysically detailed models of neuronal circuits with pharmacologically relevant parameters [10], with spatial scales from brain regions to a single synapse and time-scales spanning single action-potentials to hours or even days. Such detailed models can identify key additional data required, provided quantitative predictions and hypothesis testing [11] and potentially provide an *in silico* platform for drug discovery [12]. Computational neuroscience has traditionally built models focused on electrophysiology, synaptic signaling and corresponding network activity [13]. However, multiscale modeling for ischemic stroke must involve not only neuronal activity, but also intracellular and extracellular molecular dynamics.

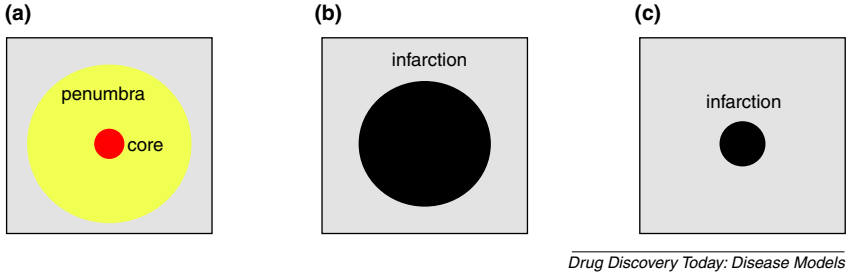

Figure 2. The goal of neuroprotection following an ischemic stroke is to reduce the tissue damage and subsequent functional impairment. There are numerous potential targets for neuroprotective therapies, across multiple temporal and spatial scales. **(a)** Simple depiction of the core and penumbra following an ischemic stroke. **(b)** A large infarct as many of the cells in the penumbra die. **(c)** A goal of neuroprotection, preserving the penumbra.

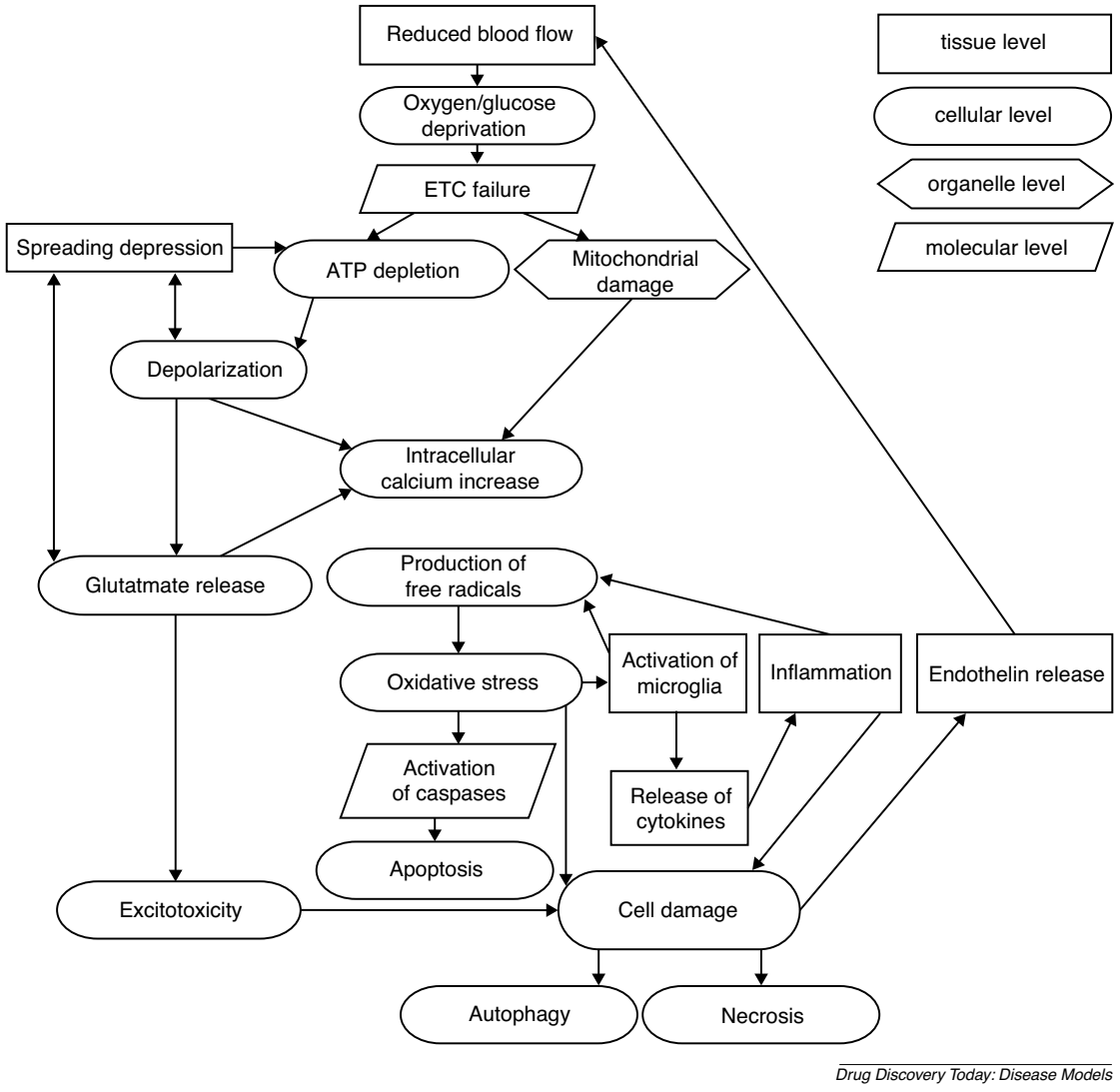

Figure 3. Schematic representation of some of the processes and pathways involved in ischemic stroke that result in cell damage or death. Multiple scales are denoted by different shapes. Several positive feedback loops amplify damaging signals and provide many potential targets for neuroprotection. ETC, electron transport chain.

Cellular scale modeling

Modeling single cells and the subcellular events that follow ischemic stroke is essential to understand the intracellular ischemic cascade, a series of biochemical reactions following ischemic insult that lead to cell damage or death. The cascade occurs over multiple time-scales, with the initial rapid changes in cell metabolism and ionic concentrations trigging several agents that may ultimately lead to cell death days or even weeks later.

There are three main modes of cell death: (1) apoptosis – programmed cell death; (2) autophagocytosis – the cell devours itself; (3) necrosis – rapid death where the products of cell death leak out to extracellular space (ECS).

There are also intermediate forms such as necroptosis. Although dead cells will be broken-down by microglia, necrotic cells are likely to cause further damage to the surrounding tissue before they are removed. The primary cause of cell death in ischemia is loss of glucose and oxygen, which reduces or prevents oxidative phosphorylation, the metabolic pathway that forms ATP. This triggers early ionic changes in the cell which in turn activate several damaging processes or perpetrators [14]. Cell death can occur within hours of ischemic stroke or weeks later. The mode and timing of cell death may not be determined by one particular perpetrator, but result from the complex interactions among multiple pathways.

A typical storm of early ischemic changes in the cell includes a decrease in pH, the production and release of free radicals, failure of the Na^+-K^+ pump due to lack of ATP, membrane depolarisation and subsequent influx of Ca^{2+}

via voltage-gated calcium channels. Depolarization leads to a rise in extracellular K^+, which may cause other neurons to depolarize in a phenomenon known as spreading depression. Influx of Ca^{2+} is augmented by calcium induced calcium release from endoplasmic reticulum. Elevated intracellular Ca^{2+} causes vesicles release, and glutamate that spills out into the ECS can extrasynaptically excite surrounding neurons. Both synaptic process and extracellular glutamate contribute to *excitotoxicity*, a neuronal damage through excessive stimulation [15]. Excitoxicity is particularly potent in the low nutrient environment of the penumbra since impaired cells cannot keep up with the increased metabolic demands. Cytotoxic edema (accumulation of water inside cells) results from osmotic movement of water into cells [16]. Astrocytic swelling reduces the volume fraction of ECS, causing further increase in concentration of damaging agents [17]. The rapid changes trigger several perpetrators, agents believed to be responsible for the functional and structural changes that lead to cell death, such as calpain (part of a family of calcium dependent enzymes that break down the cytoskeleton and may damage ion channels), and phospholipase activation that breaks down cell membranes [14]. Free radicals are a key perpetrator and include ROS and reactive nitrogen species (RNS). ROS are produced in mitochondria when the electron transport chain fails and are removed by scavenging enzymes. Oxidative stress occurs when the production of ROS exceeds the cell's ability to remove them. Nitrogen oxide (NO) is a neurotransmitter and neuromodulator; though it is necessary to maintain vascular tone and cell homeostasis, it is rapidly cleared with a half-life of a few seconds. Production of NO by nitric oxide synthase (NOS) increases ~20× in. ischemic stroke. Toxicity then occurs directly or via the formation of peroxynitrite, a RNS.

There are many models that focus on cell damage and death under a variety of pathological conditions, which are relevant to the understanding and model development for ischemic stroke. For example, in a model of mitochondria of a pancreatic β-cell, suspension of the electron transport chain (as would occur in ischemic conditions) leads to the failure of proton pump and subsequent high mitochondria membrane potential, resulting in an increase in proton leak and a corresponding increase in ROS [18]. Models of oscillations between the protein Mdm2 and gene p53 showed that Ca^{2+} induced increases NOS shift the systems from sustained oscillation to an oscillation death regime [19], emphasising the importance of considering noise in the system because of the low copy number. Detailed biophysical models of apoptosis, particularly focus on caspase, the principle signalling pathway for cell suicide. Caspase is activated by binding of surface receptors or in response to oxidative stress. Modeling has to identify factors that lead to the heterogeneity of cell fates, protein interactions that can promote or suppress apoptosis and have the potential for *in silico* drug development [20–23]. Intracellular Ca^{2+} plays a vital role in neuronal signalling and biophysiology, so it has been extensively modeled in both physiological [24] and pathological conditions [25], and it is also a principle agent in the ischemic cascade (Fig. 3), penumbra responsible for much of the subsequent toxicity. Such models are valuable for future development of multiscale models of ischemic stroke.

Tissue scale modeling

Spreading depression is a wave of depolarization that propagates through tissue at 2.5–7 mm/min and can last at a particular location for ~1 min. Spreading depression results from several causes but one that is best explored is due to the leak of K^+ from cells with spread through ECS and subsequent depolarization with further release from affected cells, with extracellular K^+ levels exceeding K^+ a threshold ~27 mM that is modulated by NO [26]. Spreading depression is seen not only in ischemic stroke, but also in migraine and epilepsy. In the latter disorders, there is sufficient energy so that the depolarized cells will repolarize once the excessive extracellular K^+ is cleared by astrocytes. In ischemic spreading depression, this clearance and repolarization will fail. Phenomenological models have attempted to capture the key events while abstracting away from the biophysical details. One model for spreading depression used functions to represent blood flow, energy (available and required), cellular damage and ability to repair, with the mode of cell death determined by simple thresholds. The model showed a correlation between the temporal frequency of spreading depression waves and the rate of expansion of the core. Repeated spreading depression waves increased core size by ~30% [27]. Mathematical modeling indicated an essential role for the Na^+-K^+ pump, which fails in ischemic causing K^+ to leak into the ECS [28] and cellular swelling, which greatly reduces the volume of the ECS while increasing the tortuosity [29].

At the tissue scale it is necessary to consider the extracellular changes in the ischemic cascade. Concentration changes of ions and molecular signals of cell damage diffuse thought the ECS at various rates influencing a large volume of tissue. Within minutes of ischemic stroke the cells at the core are fatally damaged and undergo necrosis, cells in the penumbra will also perish if not protected until blood supply is restored. Measurements from diffusion weighted MRI (DWI) indicate a rapid growth in the core over the first 3 hours after stroke [30]. Inflammation is triggered following ischemic stroke. At early stages, inflammation is harmful, increasing the concentration of free radicals and intracranial pressure. Later, inflammation may be beneficial as it clears dead cells to allow rewiring. Two key cell classes for inflammation in the brain are microglia and leukocytes. Microglia are rapidly activated following an ischemic stroke and will eliminate dead cells via phagocytosis. In doing so, they release free radicals, NO and

pro-inflammatory cytokines. Cytokines attract leukocytes from the blood. Within hours of the stroke, neutrophils enter which can be harmful as they enzymatically degrade cells. After around 24 hours, macrophages enter and perform further phagocytosis, clearing dead tissue from the infarct core. A detailed mathematical model was developed that considered the densities of these cells in different tissue states – healthy, necrotic, apoptotic and undergoing apoptosis [22]. That model revealed a nonlinear relationship between initial core volume and the ultimate size of the infarction. These data could then be used to predict the efficacy of anti-inflammatory therapy.

Ischemia triggers yet another positive feedback loop as vasoconstrictor substances such as endothelin are released and further reduces blood flow. Some models have incorporated the blood flow implicitly in terms of availably energy and oxygen [27,28]. More detailed multiscale models of cerebral blood flow have also been developed [11]. Some of these can use patient-specific computational fluid dynamics to determine regions at risk for thrombosis development [31] and could identify patient-specific drug responses [32].

Multiscale modeling for neuroprotective drug discovery

Targeted nanotherapy will provide the ability to target specific brain regions or specific cell types. Synthetic polymers have been developed for improved drug delivery. Stimuli-responsive polymers can respond in a given way in accordance with the change of cell shape, permeability or electrical properties, or in response to temperature, pH or electrical fields [33]. Given the acidification that occurs in ischemic stoke, such stimulus-response polymers have the potential to deliver therapeutic agents to particular parts of the penumbra where efficacy is predicted by detailed modeling of the pH changes expected under certain circumstances [34]. As noted above, many processes of cell death and tissue disruption associated with ischemia are highly nonlinear in both time and space. For this reason, we propose that drug delivery might best be targeted in space according to specific criteria. Although targeting drug delivery at particular delivery times may also be valuable, we note that the many processes of penumbra evolution will be distinct across the affected region so that different regions will be at different stages of evolution in the peri-infarct period. We propose this type of drug discovery as another form of medical *precision*. *Precision medicine* refers to the selection of particular groups of patients according to various grouping which often depend on the patients' genomes. *Precision drug targeting*, already used for tumors, refers to the precise targeting of particular sets of cells according to these cells' shared attributes over the course of disease progression.

Spreading depression causes cells in the penumbra to expend energy they can ill afford to waste, increasing their vulnerability to cell death. Preventing spreading depression is of interest not only to treatment of ischemic stroke, but also for migraine and epilepsy. This suggests that antimigraine drugs, which prevent spreading depression, could be tried in ischemic stroke. However, differences in the state of the tissue can substantially alter the efficacy of such a treatment so here again targeting therapy to particular states of the penumbra defined by location relative to the core or by the time after the stroke initiation could be valuable. For example, NMDA receptor antagonists are effective at limiting the waves of spreading depression in normal tissue. However, excessive extracellular K^+ is sufficient to cause spreading depression with little influence from synaptic transmission under pathological conditions [35]. Here again, precise targeting of an NMDA antagonist that depends on the details of the local tissue environment may be valuable. Computational modeling will be used to inform the relative contribution of the different causes of spreading depression, and to identify and modify the relevant parameters to provide an estimate of efficacy at particular times following the stroke, perhaps in combination with other therapeutic agents.

Many other proposed neuroprotective strategies could be assessed by modeling [36]. Examples include (1) Ca^{2+} channel antagonists [37]. Multiscale modeling could here evaluate the relative efficacy in inhibiting the different routes by which excessive Ca^{2+} can enter the cell, distinguishing pre- and postsynaptic processes. These would include presynaptic voltage-gated channels and NMDAR which would increase glutamate release, calcium-induced calcium release from organelles, stretch-activated calcium channels that might respond to the stretch associated with edema, and acid-sensing ion channel. Other factors that could be considered would be the effects of channel antagonists on blood pressure reduction. (2) Inhibiting the aquaporin-4 (AQP4) water channels [38]. Non-steroid anti-inflammatory drugs (NSAIDs) have a large number of effects which are not restricted to inflammatory agents but include effects on the aquaporin channels. This wide variety of NSAID effects have been the subject of modeling [39], which could now be extended to the special considerations encountered in ischemic brain tissue. For example, the effects on aquaporins could reduce cytotoxic edema and thereby reduce local extracellular concentrations of toxic molecules and decrease tortuosity so as to increase clearance of these molecules to sites beyond the penumbra. NSAIDs also inhibit some types of Ca^{2+} channels and could reduce in Ca^{2+} influx [39]. NSAIDs reduce prostoglandins which play a variety of roles mediated by at least 10 receptor subtypes. (3) Inhibiting leukocytes interaction with endothelial cells [40]. Inflammation is a response to injury which causes further injury, particularly in the skull-case which provides no room for expansion. General cell

density models for inflammation can distinguish between microglial and leukocyte inflammation to begin to calculate how inhibition of aggregation could affect infarct size in each case [22]. However, these models lack key features that are peculiar to the brain and to ischemic stroke, such as the effects of production and diffusion of ROS, which activate microglia and can be produced by them. The disruption of endothelial cells and of the blood brain barrier in ischemic stroke leads to particular patterns of accumulation of leukocytes such as polymorphonuclear cells. Other components of the neurovascular-unit will also contribute to immune responses following ischemic stroke.

Conclusion

Individually, several of the existing ischemic stroke models have the potential for *in silico* experiments and drug development focusing on a single aspect of stoke pathology, for example, thrombosis formation [31] or inflammation [22]. Future developments should seek to combine the multiple features together with sufficient biophysical detail to facilitate the search for multi-target drug cocktails.

Conflict of interest

The authors have no conflict of interest to declare.

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Computational Models of Neurological Disorder

Multiscale models of pharmacological, immunological and neurostimulation treatments in Alzheimer's disease

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The current study reviews computational models that embody a hypothesis and/or a theory of mechanisms of how AD impacts the brain and cognition as well as provide a critical analysis of strengths and weaknesses of these models. Existing models assume AD symptoms stem from abnormalities to cell structure, synaptic connections, neuro-chemicals, as well as other neural circuits and systems. We also discuss how mathematical formulation of the known biology of AD can help us understand AD symptoms and how pharmacological medications and neurostimulation therapies may work. Finally, we discuss general research directions that may improve future treatments of the disease.

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What is AD

Alzheimer's disease (AD) is the most common neurodegenerative aging disorder affecting millions of individuals worldwide. AD is associated with memory decline as well as impairment in language and executive function. These symptoms become more severe with disease progression. It is estimated that 25–35% of the population over the age of 85 years old have AD. The number of patients with AD is expected to rise in the near future as people are now living longer.

The formation of the beta-amyloid plaques and neurofibrillary tangles in the brains of the patients were found to be related to dementia symptoms [1]. It is not known which factors lead the formation of plaques and tangles in some individuals and how exactly they relate to different symptoms in AD. In addition, several neuropsychological and fMRI reports show hippocampal dysfunction in AD patients [2–6]. Current studies attempt to develop deep brain stimulation therapy for AD targeting different hippocampal regions, including the hippocampus, fornix, and entorhinal cortex [7,8].

It has been found that variations in apolipoprotein E (APOE) genotype are associated with increased risk of developing AD [9]. There are three different genetic alleles that encode the APOE gene: ε2, ε3, and ε4. Approximately, 15% of the population carry the APOE ε4 allele, while the rest carry the APOE ε2 or APOE ε3 allele. Importantly, APOE ε4 has been linked to AD pathology more than the other

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alleles. Along the same lines, carriers of the APOE ε4 genotype have been shown to have larger temporal lobe atrophy (a brain area implicated in AD) and poorer memory functions than non-carriers [10]. Similarly, it was found that APOE ε4 allele is associated with a small hippocampal volume in healthy older subjects. Further, studies have also reported reduced acetylcholine levels in the hippocampus in AD patients [11].

AD etiologies

The etiology of AD is very complex and several hypotheses have been put forward to explain the pathogenesis of the disease. Below, we describe some of the most prominent hypotheses and present experimental supporting evidence for each one.

Amyloid hypothesis

The amyloid hypothesis proposes that extracellular beta-amyloid (Aβ) plaques are the fundamental cause of the disease [12]. Aβ is a fragment of a transmembrane protein that penetrates through the neuron's membrane, the amyloid precursor protein (APP). In AD, APP is divided into smaller fragments by proteolytic enzymes including β-APP cleaving enzyme (BACE) [13–15] and γ-secretase [16,17]. One of these fragments (39–43 amino acids in length) form dense formations (Aβ plaques) in the extracellular space of neurons. Amyloid hypothesis is further supported by the discovery that AD could also be caused by autosomal dominant mutations in presenilin 1 (PSEN1) [18] and PSEN2 [19,20], which are both homologous proteins that can form the catalytic active site of γ-secretase. As mentioned previously, the APOE gene represents the major genetic risk factor for AD [21,22] with the possession of the APOE4 allele speeding considerably the age of disease.

Cholinergic hypothesis

The cholinergic hypothesis proposes that memory deterioration observed in AD patients is caused by a reduced synthesis of acetylcholine (ACh), choline uptake, and ACh release [23]. The correlation of deficits in brain cholinergic system and AD symptom severity is supported by various cell culture and animal model studies that show a central role of ACh regulating amyloidogenic processing of APP and hyperphosphorylation of tau.

Tau hypothesis

The tau hypothesis [24] proposes that hyperphosphorylated tau proteins form neurofibrillary tangles inside the nerve cell bodies, causing microtubules to disintegrate, collapsing the neuron's transport system. As Aβ plaques and neurofibrillary tangles accumulate in the brain, synaptic and neuronal losses occur on a large scale affecting the entire cerebral cortex, the hippocampus and neighboring brain regions.

Glucose synthase kinase 3 (GSK3) hypothesis

According to this hypothesis, over-activity of GSK3, a proline-directed serine/threonine kinase, accounts for memory impairment, tau hyper-phosphorylation, increased Aβ production, reduction of ACh synthesis, cell apoptosis, and local plaque-associated microglial-mediated inflammatory responses, all of which are principal characteristics of AD [25].

Oxidative stress hypothesis

According to this hypothesis, increased oxidative stress leads to AD [26]. Increased oxidative stress in the brains of AD patients is correlated with increased levels of free radicals and metals (iron, copper, zinc, and aluminum), increased inflammatory response from activated microglia and astroglia, and increased levels of advanced glycation endproducts (AGE). The imbalance between the generation of free radicals and age-related accumulation of reactive oxygen species (ROS) results in a damage to major components of cells: nucleus, mitochondrial DNA, membranes, and cytoplasmic proteins. Aβ has been found to be sensitive to the action of free radicals, contributing to aggregation and itself producing peptides in free radical form [26]. APOE is subject to free radical attacks, and APOE peroxidation has been correlated with AD [26].

Each of these cascades produces *secondary effects* to the nerve cells which may result in cell death [27], synaptic loss [28], alterations of ionic and synaptic channels [29], impairments in synaptic transmission and plasticity [30], destabilization of neural network activity [31,32], aberrant network synchronization [32], alterations in microglia response [33], or CREB down-regulation [34] throughout the cerebral cortex and hippocampus.

AD therapies

There are many pharmacological medications approved for AD, including donepezil, galantamine, rivastigmine, and memantine. Some of these pharmacological agents (donepezil, galantamine, rivastigmine) are cholinesterase inhibitors and thus increase acetylcholine levels in the brain, while memantine is an NMDA antagonist. Memantine was shown to increase acetylcholine (ACh) levels in the hippocampus, but it does not improve memory performance in rats [35]. This is in contrast to studies showing that ACh inhibitors increase ACh levels and also improve memory function in animal models and patients with AD. Howard and colleagues [36] have found that donepezil or memantine are effective for enhancing memory in moderate-to-severe AD patients, although adding both together does not lead to any additional improvement. One major problem with currently approved AD drugs (ACh inhibitors and NMDA antagonists) is that they are symptomatic and work for a short period of time.

Alternative forms of treatment to pharmacoresistant AD patients are electrical stimulation techniques including

transcranial electrical nerve stimulation, radioelectrical asymmetrical brain stimulation, vagal nerve stimulation and deep brain stimulation. Transcranial electrical nerve stimulation of hippocampal structures has been associated with memory improvement in patients with AD [37]. Vagal nerve stimulation improve cognitive outcome in AD patients [38]. Radioelectrical asymmetrical brain stimulation using radiofrequency bursts have shown improvements in MMSE (Mini Mental State Examination) scores in AD [39]. Clinical DBS stimulation studies in the fornix, entorhinal cortex, hippocampus and nucleus basalis of Meynert have shown that DBS has the potential to enhance memory function in human patients and animal models [7,8,40–42]. Suthana and colleagues [8] have shown that stimulation of the entorhinal region enhanced spatial memory when applied during learning. Toda and colleagues [42] have shown that electrical stimulation can enhance neurogenesis in the hippocampus. Despite these electrostimulation memory enhancement and restoration, the nature of the stimulation-induced modification of the neural circuits that result in memory improvement in AD patients is still not completely understood.

Computational multiscale models of AD drug discovery and treatment

As we described above it is experimentally very difficult to understand how the interactions of the various molecular pathways and mechanisms lead to the pathogenesis of AD and its symptoms. Equally difficult are the various potential routes of cure by drug and electrostimulation therapies. This is mainly because experimental studies are usually carried out to isolate the effects of a single mechanism and do not investigate the interactions of many mechanisms. This leads to a set of results that are conflicting, very difficult to interpret, or not integrated in a unified framework.

Mathematical and computational models are invaluable tools in resolving such conflicts, because they provide coherent conceptual frameworks for integrating many different spatial and temporal scales and resolutions that allow for observing and experimenting with the neural system as a whole. Computational modellers then have precise control of experimental conditions needed for the replicability of experimental results. Because the process takes place in a computer, the investigator can perform multiple virtual experiments by preparing and manipulating the system in precisely repeatable ways and observe every aspect of the system without interference.

In the next section we will describe computational multiscale modelling attempts ranging from molecular and biochemical level to neural circuits and systems level of AD pathogenesis and pharmacological, immunological and neurostimulation treatments.

Multiscale models of pharmacological and immunological therapies

Molecular and biochemical models

Early mathematical and computational biochemical modelling of AD focused on the amyloid β (Aβ) fibrillogenesis, a key defining pathological feature of AD. As mentioned before, Aβ is a fragment of a transmembrane protein that penetrates through the neuron's membrane, the amyloid precursor protein (APP). In AD, APP is cleaved into smaller fragments by proteolytic enzymes including α-, β- and γ-secretases and produce Aβ plaques in the brain. It was hypothesized that secretase inhibitors can reduce the production of Aβ in the brain and thus may slow the progression of AD. Paradoxically, it has been shown that low to moderate inhibitor concentrations cause a rise in Aβ production in different cell lines, in different animal models, and also in humans. Ortega and colleagues [43] developed a minimal mechanistic understanding of Aβ dynamics in cell lines that either exhibit the rise or not. They showed that the cross-talk between the amyloidogenic and the non amyloidogenic pathways accounts for the increase in Aβ production in response to inhibitor (C99) redirecting this way APP to be cleaved by β-secretase, leading to an additional increase in C99 that overcomes the loss in γ-secretase activity. The model had a widespread impact on the development of drugs targeting Aβ production in AD. It could be used to form decisions about *in vitro* cell lines and *in vivo* models used in drug discovery studies. It could also be used to investigate the implications of alternative therapies, such as β-secretase inhibition or α-secretase promotion, as well as combination therapies.

Others investigated more complex factors and processes that may disrupt Aβ regulation. Anastasio [44] computationally demonstrated how incipient cerebrovascular disease (CVD), inflammation and oxidative stress (OS) can be such pathological processes. Particularly he suggested treatments directed at multiple targets can be more effective than single target therapies. In another study, ways by which estrogen therapy might be used more effectively in AD treatment, perhaps by administering estrogen in conjunction with other agents were explored [45]. Under conditions of very low estrogen and incipient CVD, the level of Aβ could be reduced, possibly to normative levels, with a combination of a nonsteriodal anti-inflammatory drug (NSAID) that promotes peroxisome proliferator-activated receptor (PPAR) expression, a compound that blocks hypoxia inducible factor (HIF), and estrogen itself. The model suggested that estrogen would provide the main benefit, reducing Aβ directly (e.g., by enhancing neprilysin (NEP) expression) and indirectly by reducing inflammation and OS (e.g., by enhancing superoxide dismutase expression), thereby disrupting pathological processes that contribute to Aβ accumulation. With estrogen itself providing the main benefit, an NSAID and a HIF-blocker

can each provide a small additional benefit, and these two benefits are additive in combination.

Another defining characteristic of AD is the dysregulation of synaptic plasticity by Aβ. In the normal synapse where Aβ is absent, PKA is responsible for keeping striatal-enriched protein tyrosine phosphatase (STEP) (and other key LTD drivers) inactive when Ca^{2+} is high enough to elicit LTP. On the other hand, in the diseased synapse where Aβ is present, the action of PKA is instrumental in preventing LTD from occurring at all non-zero levels of presynaptic activity including that which would evoke LTP in the normal synapse. PKA is thus the mediator that keeps the diseased synapse at least at baseline at high levels of presynaptic activity. Anastasio [46] provided an initial modeling framework for understanding how various drugs and drug combinations might operate in the diseased synapse. It suggested that normalization of nicotinic acetylcholine receptors (nAChR) function may be the most effective way to counteract the adverse effects of Aβ on synaptic plasticity, lending some modelling support to the suggestion that disordered nAChR function is the main route by which Aβ dysregulates synaptic plasticity [47].

Immunotherapy against Aβ has recently been shown to be more effective when it is applied to in the early stages of the disease. The effects of passive and active immunization on soluble Aβ, plaques, phosphorylated tau and tangles showed that Aβ clearance proceeds into steps with the administration of antibodies and microglia. Proctor and colleagues [48] modelled the effects of immunotherapy by adding a species named 'anti Aβ' to represent the addition of antibodies (i.e. passive immunization) and another species named 'Glia' to represent microglia. The addition of antibodies and microglia were done at predetermined time points during the simulation. The aggregation process started with the formation of Aβ dimmers from two monomers, but this reaction was reversible. Under normal conditions, model Aβ levels started at very low values and Aβ was continually produced and degraded. The model predicted that immunization leads to clearance of plaques, but has small effect on soluble Aβ, tau and tangles.

Treatment combinations of 10 FDA approved drugs (auranofin, bortezomib, dasatinib, glimepiride, ibuprofen, naloxone, nicotine, rosiglitazone, ruxolitinib, and thalidomide) were investigated in reducing microglial inflammation in AD [49]. Out of the 1024 possible drug combinations, simulations identified only 7 combinations of the auranofin, glimepiride, ibuprofen, rosiglitazone, nicotine and naloxone drugs were able to reduce microglial inflammation in AD. Analysis showed that out of the 7 most efficacious combinations, the 'glimepiride/ibuprofen' and the 'glimepiride/ibuprofen/nicotine' administrations stand out as superior both in strength and reliability to completely reverse the neurotoxic effects of AD inflammation.

Neural circuits and systems models

Essential steps in drug discovery and therapy of neurodegenerative disorders such as AD are the development of computational models that bridge the gap between behavior, cellular physiology and molecular biology in the study of human memory. The effects of scopolamine, a drug that blocks the cellular effects of acetylcholine, were investigated in the encoding and retrieval of memories in a cortico-hippocampal model (EC-Dentate gyrus-CA3-CA1) [50]. 'Memory' was represented as a pattern of neural activation in each module, with information flowing from EC to DG to CA3 to CA1. Simulations showed that scopolamine blockade of ACh impaired the encoding of new input patterns, but had no effect in the free recall of input patterns learned before the blockade. This was due to scopolamine blocking the strengthening of recurrent connections in region CA3 to form attractor states for new items (encoding impaired), while allowing recurrent excitation to drive the network into previously stored attractor states (retrieval spared). Despite its successes, the model's network dynamics were based on abstract formulations of cells and their interactions. Furthermore, it failed to consider the cellular consequences of ACh in the intrinsic cell firing.

The differential effect of memantine, an NMDA inhibitor, in early and late AD pathology was examined by a biophysically realistic model of cortical circuitry simulating working memory as a measure for cognitive function [51]. The pathology of AD was implemented as synaptic and neuronal loss and a decrease in cholinergic tone [51]. The model was subsequently calibrated using preclinical data on receptor pharmacology of catecholamine and cholinergic neurotransmitters [51]. Simulations showed that inhibition of the NMDA receptor NR2C/NR2D subunits located on inhibitory interneurons compensated for the greater excitatory decline observed with AD pathology [51]. Like any other model, the Roberts' model was also bounded by limitations including the relatively low number of neurons used and the rather simple morphological representation of excitatory and inhibitory cells in the network.

Pharmacological manipulations of experience-dependent activation of specific transcription factors (e.g., cAMP Response Element Binding protein (CREB)) and their resulting gene alterations have suggested improvements of memory impairments due to AD [52,53]. Experimental work in rodent hippocampus has shown that CREB activity increases in regions CA1 or dentate gyrus memory formation, storage and recall, enhancements and restorations [54,55]. Inspired by the modeling work of Cutsuridis and colleagues [56], Bianchi *et al.* [57] investigated the conditions in the CA1 microcircuit under which the properties of pyramidal neurons altered by increasing CREB activity can contribute to memory storage and recall improvements. The effects of CREB were modelled as decreases in the peak conductances

of mAHP and sAHP currents by 52% and by 64% respectively and an increase in the peak AMPA conductance by 266%. With a set of patterns already stored in the network, they found that the pattern recall quality under AD-like conditions (i.e. when the number of synapses involved in storage is reduced and/or the peak AMPA conductance is reduced) is significantly better when boosting CREB function. They inferred that the use of CREB-based therapies could provide a new approach to treat AD.

Models of neurostimulation therapy

As AD progresses, cells die and synapses lose their drive, causing the remaining cells in the network to suffer an initial decrease in activity due to homeostatic synaptic scaling. This homeostatic mechanism is believed to sense levels of activity-dependent cytosolic calcium within the cell and to adjust neuronal firing activity by increasing the density of AMPA synapses at remaining synapses to achieve balance. The scaling mechanism increases the firing rates of remaining cells in the network to compensate for decreases in network activity. However, this effect can itself becomes a pathology as it produces increased imbalance between excitatory and inhibitory circuits, leading to greater susceptibility to further cell loss via calcium-mediated excitotoxicity. Rowan and colleagues [58] advanced a mechanistic explanation of how directed brain stimulation might be expected to slow AD progression based on computational simulations in a 470-neuron biomimetic model of a neocortical column. The simulations demonstrated that therapeutic low-intensity low-frequency electro-stimulation could act on homeostatic synaptic scaling mechanisms to reduce the pathological effect of excessive compensatory scaling in AD disease. The increase in activity within the remaining cells in the column results in lower scaling-driven AMPAR up regulation, reduced imbalances in excitatory and inhibitory circuits, and lower susceptibility to ongoing damage.

Conclusions and future work

We have provided here the first extensive review of computational multiscale attempts of AD pathogenesis and treatment ranging from molecular and biochemical to neural circuits and systems level. The models embodied a hypothesis and/or a theory of mechanisms of AD. Each model's main computational elements are highlighted and a critical analysis of each model's strengths and weaknesses is also provided. Existing models assume that AD symptoms stem from abnormalities to molecular pathways, cell structure, synaptic connection, neurochemicals, as well as other neural systems.

We suggest below general research directions that were omitted from the current models in order to improve future treatments of the disease. Future models should include sufficient details on the structural and functional subunits of the neurons, so they are able to simulate molecular effects of pharmacological treatments (e.g., effects of drugs on receptors and dendrites).

Furthermore, future models should explain the relationship between neural changes (formation of plaques and tangles, reduction in Ach levels) to behavioral symptoms (memory decline, semantic memory deficits, executive dysfunction) in AD. They should focus on simulating memory decline as well as simulate other AD neural and behavioral abnormalities such as executive dysfunction or apraxia.

Moreover, future models should simulate the effects of medications (donepezil, galantamine, rivastigmine, and memantine) on neural and behavioral processes. They should explain how increasing Ach levels and NMDA antagonists does relate to memory improvement as well as how they affect other symptoms of AD. Further, although most (if not all) of the neural and behavioral studies differentiate between mild-to-moderate vs. severe AD patients, and also whether patients are APOE ε4 carriers or not, computational modelling studies should address these subgroups of AD patients.

Finally, as many experimental studies explore the benefits of deep brain stimulation therapy for AD [7,8], it is expected that computational models will be needed to explore how it works and best ways to find best deep brain stimulation parameter values for frequency, pulse width, and voltage (as well as locations within the hippocampal region) to treat AD. Such work can greatly benefit from existing models of deep brain stimulation applied to Parkinson's disease [59,60], to explore how it may reduce AD symptoms.

Conflict of interest

The authors declare that they have no competing financial interests.

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