A systems approach to Parkinson's disease

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Abstract

We describe a systems approach to Parkinson's disease in which an integrative mathematical model of the brain energy metabolism is used as the basis for *in–silico* investigation of possible disease mechanisms. The mathematical model allows both the analysis of dynamical and structural implications of biological factors implicated in disease causation, and the potential role of a compromised brain energy metabolism as factor that enables pathologies associated with the disease to develop. Using a recently developed mathematical model of brain energy metabolism, we use simulations of brain energy behaviour to illustrate how a weakened energy metabolism might create a cellular environment where pathogenic mechanisms that are normally held in check by an efficient energy metabolism could potentially flourish. Simulations of the brain energy metabolism are presented which illustrate how both very long term deterioration of metabolism, and cumulative stress from energy transients, may create a predisposition to Parkinson's disease. The focus of this study is Parkinson's disease, but we note that our energy metabolism modelling and analysis framework may also form a basis for a systems approach to other age–related neurodegenerative conditions.

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Introduction

Mathematics in biology

Treating known (or hypothesized) biological mechanisms from a mathematical approach has been used with great success in biology. One of the most widely known examples is the mathematical description of enzyme activity by Michaelis and Menten [35], with further developments by Briggs and Haldane [10]. This kinetic equation is an elegant way of describing enzyme action – a biological system comprising of hundreds of molecules – using a mathematical expression with just two parameters. The success and wide application of this equation makes it clear that it is possible to use mathematical tools to organise biological knowledge efficiently. For example, when scientists want to compare isoforms of an enzyme, the Michaelis and Menten parameters (maximal activity and affinity) provide the objective and quantitative information with which to perform the comparison. The idea that biological systems that are larger and more complex than an enzyme can also be characterised by mathematical equations (e.g a mathematical model) is the basis of the field known as systems biology.

The systems approach to biology carries a number of potential advantages. For example, using a mathematical model a biological process can be studied rapidly, in detail, with little cost and without access to the actual system. Likewise, possible variations on a biological system can be analyzed in a wide variety of configurations in a precisely reproducible way. A mathematical model can also be used to observe and analyze the behaviour of variables that cannot be measured experimentally in a biological process. The behaviour of these 'hidden' system states can be crucial to understanding the performance of biological systems where measurement is difficult or impractical. Finally, the implications of specific combinations of events that occur over the entire lifetime of an organism can be investigated rapidly and in a repeatable manner. These last two points are of particular relevance to the study of diseases. And especially to degenerative diseases of age, such as Parkinson's disease, that are unique to the human brain and for which animal models reproduce only certain pathological features.

A systems approach to neurodegenerative disease

In order to be relevant, a systems approach to disease should be strongly based on what we know about physiology and metabolism of the affected tissues. In the case of the human brain, and despite the fact that it is one of the most complex tissues, a considerable amount of data and hypotheses have been generated concerning how it functions and how it malfunctions. As cerebral systems are complex and the data sources are diverse, it is hard to integrate this knowledge and produce predictions and further hypotheses (e.g. *is gene X relevant in disease Y?*). We thus argue that the time is ripe to perform this integration of biological knowledge with the aid of descriptive mathematical modelling and analysis of the neurodegenerative mechanisms. In particular, we take techniques of the kind used to analyse the behaviour of complex technological processes and apply them to

failures in the brain. The approach is based upon a combination of mathematical modelling, computer (*in–silico*) simulation and mathematical analysis of the model dynamics.

Our approach is based on a mathematical description of the bio-chemical kinetics of the brain energy metabolism, and which provides the modelling framework for a systems approach in which the system studied can be extended by the addition of sub–models of any relevant biological process. In this way we have an integrative mathematical modelling approach to disease that is capable of incremental expansion by the addition of sub-models of cellular process thought to be implicated in a particular pathology. When implemented *in–silico*, the resultant mathematical model allows the simulation of disease conditions and the analysis of their behavioural/structural properties. We emphasise however that this position paper focusses upon the general features of, and justification for, a systems approach which includes the potential role of brain energy metabolism in Parkinson's disease – the incorporation of sub–models within the framework will be the subject of further work.

Why Parkinson's Disease?

The choice of brain energy metabolism as the basis for a systems approach to Parkinson's disease was driven primarily by mathematical modelling and systems analysis considerations. However, we believe that the modelling of energy metabolism may also have value in the exploring how a compromised energy metabolism could facilitate age–related neurodegenerative disorders. Such an approach is of particular relevance to the most prevalent idiopathic form of Parkinson's disease(iPD), because of its apparent multi-factorial nature. Specifically, although a number of genetic, cellular and environmental factors have been linked with idiopathic PD [17, 46], a unique causal mechanism has yet to be identified. In fact, the weight of evidence suggests that iPD is the composite result of a range of possible factors [29]. Individually these factors maybe benign; only when a number of them coincide in conditions favourable to disease do they trigger iPD.

We hypothesize that favourable conditions for iPD maybe created by metabolic weaknesses that develop over the lifetime of an organism [39]. In this context we note that advanced age is the key common factor in patients with idopathic PD [18]. With age comes a deterioration of energy metabolism [49] and, as will be described later, the cells most vulnerable to iPD are those with the highest energy requirements. Indeed, there is evidence of a scale of vulnerability, in which the higher the energy budget, the higher the susceptibility to iPD damage. Based upon these observations, we suggest that a brain energy metabolism compromised by age, or otherwise damaged during a lifetime, may create deficits in cellular function that permit pathogenic mechanisms to flourish.

The idea that iPD is due to the coincidence of a range of possible causal mechanisms that can occur in a range of combinations is given weight by recent medical opinion which comes to a similar conclusion [27], [28]. In the words of a recent survey article on the pathogenesis of PD [29], such a possibility is '*bad news for scientists*

hoping to find one single cause of idiopathic Parkinson's disease.'

In-vivo, *in-vitro* and *in-silico* models

The likelihood of a multi–factorial basis for iPD is bad news indeed for traditional medical and biological research methodologies. For if iPD is due to combinatorial failures from a set of possible implicated factors, then this massively complicates the planning and execution of *in–vivo* and *in–vitro* experimental studies. The additional point that the disease progression involves changes unique to humans and that develop over the entire lifetime a brain, adds to the bad news.

In addition, the importance of dynamical change and temporal progression in understanding disease processes is often neglected. Proteomic surveys of the relevant human brain areas [24] offer valuable insights, but they are static insights that give snapshots of disease factors at a particular time. But PD is a dynamic process and time histories (both short-term transient and long-term trend) are needed to understand how a system such as the living brain arrives at a particular state.

The bad news alluded to earlier is not necessarily so bad if we consider how dynamical modelling and systems analysis techniques might help. Specifically, an *in–silico* model of disease dynamics can be run as many times as required, in any multifactorial combination, much faster than the chronological time needed for the corresponding biology to unfold and with complete control over the simulated experimental conditions. The issue of chronological time is particularly relevant to neurodegenerative diseases of age – such as Parkinson's disease – where structured *in-vivo* multifactorial studies of ageing effects in human subjects present huge logistic and technical obstacles.

In addition to multifactorial issues associated with disease causation, there are also constraints upon the validity of *in-vivo* animal studies [40, 4] and cellular homologues [5]. Animal models of PD only reproduce certain symptomatic features associated with the disease, and the translation of results from cellular models to the *in–vivo* human case is frequently questioned. Similar comments apply to *in–vitro* studies of Parkinson's disease, since removing cells from their normal environment changes the way in which they behave. Neurons are intimately linked to their astrocytic support structures and the brain blood capillaries; they can be removed from this natural environment and studied *in–vitro*, but the system that is studied will be different and will behave accordingly.

Against this background, we argue that an integrative mathematical model of Parkinson's disease, implemented *in–silico*, represents a valuable additional research tool for investigation of disease causation and progress. A tool that can allow research results to be integrated into a system model and tested in a way that complements conventional *in–vivo* and *in–vitro* research. Such an approach avoids issues of subjectivity and repeatability, and can be used to explore speculatively the role of putative disease mechanisms faster and cheaper than laboratory

based research.

The mathematical modelling of disease in the form proposed here does not replace the ground–truth offered by biological or clinical discovery. However, a systems approach based on an integrative mathematical model offers a quantitative repository of knowledge within which all biological factors in a multi-factorial disease can be objectively analyzed and assessed. Such assessments provide intelligent guidance for the discovery process. The aim of this position paper is to indicate how such a systems approach to neurodegenerative disease can be formulated. We do this by focussing upon the choice and construction of the appropriate mathematical model and the potential implications of a compromised brain energy metabolism for iPD.

Layout of the paper

The material is laid out as follows. First we discuss in general terms how an integrative systems approach to Parkinson's disease can be made. In particular, we note that the mathematical equations which govern energy utilisation are the basis for modelling and analysis of the performance of physical systems. It is then argued that energy flow performs a similar role in living systems, with the equivalent process being the energy metabolism, and that a mathematical model of brain energy can form the framework for a systems approach to Parkinson's disease.

We then describe the circumstantial evidence that links advanced age, together with high energy requirements of a cell, to increased vulnerability to iPD. These observations are used to support the hypothesis that the mechanisms implicated in Parkinson's disease may be intimately associated with the energetic processes within vulnerable cells, and that the long term deterioration of the brain energy metabolism itself may explain why advanced age is the key factor common in idiopathic Parkinson's disease.

As a demonstration of these arguments we describe the development of a mathematical model of brain energy metabolism. A computer implementation of the model is then used to illustrate the integrated nature of energy interchanges that support neuronal energy demands, and to emphasize the importance of transient energy demands. Finally, we demonstrate how *in–silico* simulation can be used to explore ways in which long term factors might influence the progression of iPD.

A Systems Approach to Parkinson's Disease

A multi-factorial view of Parkinson's disease

An important reason for a systems approach is the probability that iPD does not have a unique cause, rather a set of possible contributory factors that can trigger the disease when they coincide in suitable circumstances.

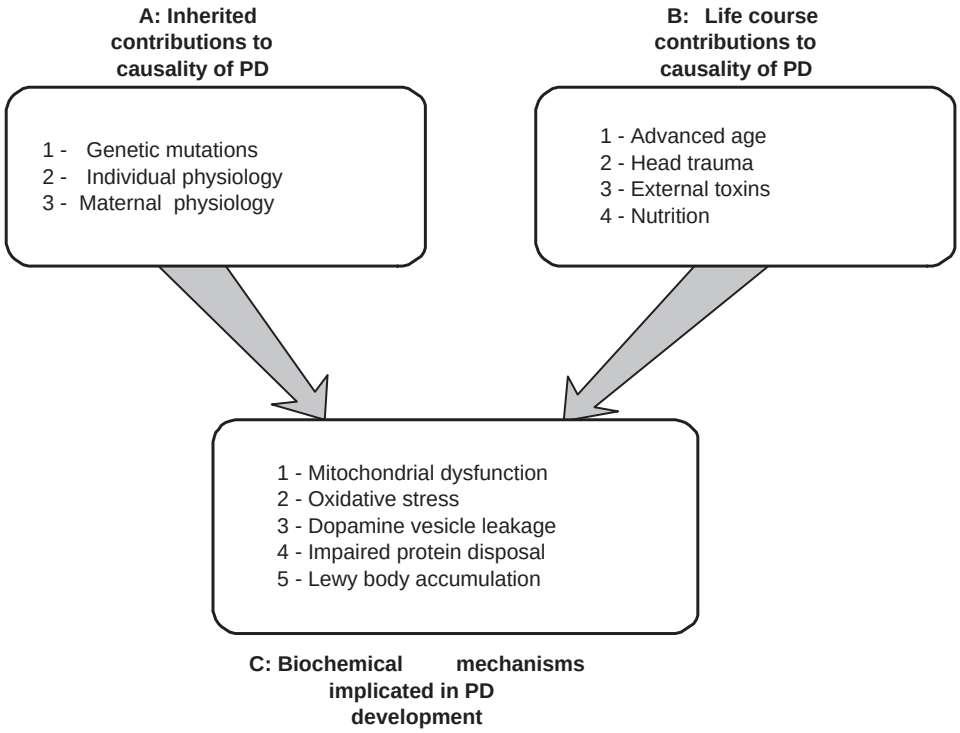

Figure 1: **A multi–factorial view of Parkinson's disease.** The various potential contributory factors are divided into three categories: (A) Inherited factors, (B) those associated with age and lifetime experience and (C) biochemical cellular processes that have been associated with iPD. We propose that iPD may be caused by any one of a number combination of processes from category C, but enabled by items drawn from categories A and B. In the energy theory for iPD, elements from category B would primary enabling events – in particular a compromised brain energy metabolism associated with advanced age or head trauma.

Three classes of possible contributory factors are illustrated in Figure 1. They are divided into, those based on inherited features, another set based upon life–course experiences and a third that covers flaws in normal cellular functions. Thus we have: (A) what we are born with (the initial conditions), (B) what happens to us (the life-time experience) and (C) their interaction with biochemical mechanisms in the cell that have been implicated in the PD pathology [32], [15], [11]. Items A and B in Figure 1 are associated with etiological factors that create a predisposition to the disease. The pathological mechanisms in C are representative of issues cited as implicated in Parkinson's disease – they are not definitive lists.

As explained below, we argue that a number of combinations of the factors in Figure 1 can form a causal pathway to Parkinson's disease. We further suggest that the wide variation in symptoms associated with iPD supports the proposition that there is not necessarily a fixed pattern of causality for iPD, and that a spectrum of causal mechanisms may exist – corresponding to the spectrum of symptoms experienced by sufferers. With this multi–factorial viewpoint of disease causation, our aim in this article is to describe a systems approach to Parkinson's disease that can be used to study the various possible disease mechanisms *in–silico* and offer a means of navigation through the multiplicity of possible causal factors.

Given the multi–factorial view of disease causation, the initial question poses itself as: *Is there a common starting point for a system approach to Parkinson's disease?* For us as systems analysts, the starting point is to use the known shared precondition for iPD – advanced age – to guide us toward the upstream common factors. In particular, we will use an energy systems model as a vehicle for showing how flaws in brain energy metabolism (from block B in Figure 1 and possibly facilitated by elements from block A) may create a predisposition to disease that enables elements in block C to take hold.

The role of modelling in systems analysis

The starting point for a systems approach to any process is the construction of a mathematical model that describes the dynamical and analytical properties of the process as it operates in its normal environment. The dynamical properties describe how the system behaves over time, while the analytical properties of a mathematical model permit theoretical aspects of a system's performance, such as stability and robustness, to be studied [44]. Changes in the system performance can reflect directly upon potential disease states [23]. When implemented in a computer, a mathematical model's behaviour permits interactions between various parts of the system to be studied objectively, rapidly and in an integrated manner that is not possible in a wet laboratory or clinical trials. As noted previously the ability to integrate various disease factors, and to efficiently test interactions between them, will be needed if we are to understand iPD from a multi–factorial standpoint.

An additional advantage of a systems approach, and one common in technological problem–solving using mathematical models, is that by embedding all potential causal factors within a mathematical model, questions

of causality can be analyzed. As Gibson [20] remarks, the chicken/egg debate over causality is a feature of neuropathology. However, by using the mathematical tools associated with a systems approach we are able to identify the feedback phenomena that lie at the heart of causality conflicts, and thus resolve the debate in an analytical manner.

Energy as a basis for modelling Parkinson's disease

Since the Newtonian era, mathematical expressions that are quantitative models of observed physical phenomena have been fundamental to understanding how physical and engineering systems work. In the last hundred years we have learnt how energy flow and storage play a central role in determining the dynamical behaviour of systems. In particular, the behaviour of all physical systems can be understood from a consideration of (i) the energy handling properties of its component parts, (ii) the ways in which the pattern of component interconnection integrates and distributes energy throughout the system and (iii) the properties of control systems that modulate energy flow [37], [31], [47].

Given that energy flow is a unifying basis for physical system modelling, it a natural scientific extension to use energy as a unifying basis for the modelling and analysis of living systems. However, there is also a compelling biological reason for using an energy principle for modelling neurodegenerative disorders such as Parkinson's disease. As we have noted previously, advanced age is the key common symptom in idiopathic Parkinson's disease. If we associate this with the observation that both the cerebral energy metabolism [49], and the effectiveness with which we ultilise glucose [39], decline with age, then a connection can be made between iPD and a compromised energy metabolism.

Significant circumstantial evidence (listed below) exists which points to compromised brain energy metabolism as an upstream facilitating factor in the etiology of idiopathic Parkinson's disease. Specifically:

1. Only cells in the nervous system are damaged in Parkinson's disease. These cells also have a unique energy metabolism, one that is specially adapted for the high energy turn–over and frequent transient energy demands required in the brain. It consists of an integration of astrocytic cells and neurons, in which astrocytes have been recruited to assist neuronal energy metabolism during rapid energy demands [38].

2. In the same spirit, combined with frequent transient energy demands, neurons in general have an exceptionally high quiescent energy turn-over rate, accounting for 20 percent of the body's rest metabolism [2]. This high metabolic rate means that neurons are more susceptible to failure if their metabolic energy control systems are compromised. Similar energy stressing might develop in cells other than neurons, but at a lower rate. Moreover, most other cells are capable of division and thus have protective mechanisms not available to neurons [29].

3. Dopaminergic neurons in the substantia nigra (SN) are the most vulnerable to damaged in Parkinson's disease. These are also the most energy demanding neurons in the brain, with (in the rat brain) an estimated number of 370,000 synapses per dopaminergic neuron, compared to estimates in the thousands of synapses for (glutamatergic) neurons elsewhere [34]. This would suggest an energy budget for SN dopaminergic neurons that is very much greater than elsewhere in the brain – possibly two orders of magnitude greater. Axonal extensions of SN dopaminergic neurons are also longer and more complex [34], with a concommitantly larger energy requirement for signalling [2] [36] which would increase the energy requirement SN dopaminergic cells yet further. The validity of the claim for such remarkably high relative energy requirement is subject to an number of assumptions: (i) energy requirements of dopaminergic and glutamatergic neurons are broadly similar. (In [2] glutametergic cells were considered). (ii) The firing rate of SN dopaminergic neurons is in the same range as that assumed in [2]. (Results in [48] place the dopamine firing rate in the middle of the firing rate in [2]). (iii) That estimates of energy consumption obtained for the rat brain translate in the human case.

4. The energy budget in SN neurons is also extremely high because of their regular pacemaking activity using an L-type calcium channel [12]. The particular energetic stress of autonomous spiking activity that strongly depends upon calcium channels has been remarked upon [11].

5. Neurons with large energy requirements elsewhere in the brain are also vulnerable to Parkinsonian damage. In particular, cells with long lightly myelinated axons appear preferentially susceptible [7].

6. Damage by environmental toxins [46], [26] or oxidative stress to organelles central to energy metabolism – the mitochondria – is known to be associated with PD symptoms. We also can anticipate that oxidative stress will be higher in brain regions with greater synaptic abundance – such as the dopaminergic neurons of the SN.

7. Plausible connections have been made between head trauma earlier in life and a predisposition to iPD [6]. As illustrated later, astrocytic/capilliary support systems compromised by head trauma, may reduce the efficiency of the metabolic systems that regulate and control brain energy supplies with potential long term effects on cellular function.

8. The accumulation of alpha-synuclein in Parkinsonian brains suggests inefficiencies/failures in the protein disposal mechanisms. While genetic reasons have been posited for this [16], our multifactorial view suggests that additional contributory factors could be the high energy demands that exist within neurons and the specific role of alpha-synuclein. In particular, it is plausible that neurons might selectively neglect protein disposal functions if they have weak energy metabolism and experience high demands from more essential cellular functions. The retention of alpha-synuclein due to inefficient brain energy metabolism would

have particular implications for dopaminergic cells [29] in the SN where (as indicated above) the energy requirements are higher than elsewhere in the brain. Alpha-synuclein is also involved in vesicle binding [29], so that dopaminergic neurons in the SN, with their high signalling budget would potentially be the neurons with the highest turnover rate of alpha-synuclein. Thus, in the case that the clearance of damaged proteins is impaired, the accumulation of damaged alpha-synuclein and incidental damage would be first observed in dopaminergic neurons. As a related remark, it has been shown [25] that mice genetically engineered to remove neuronal macroautophagy develop symptoms of neurodegeneration.

9. Finally, the energy argument can be linked to Braak's staging hypothesis for PD [8, 9]. The high energy demands of neurons with long axonal projections to the enteric system (the starting point in the staging process) would make them preferentially susceptible to energy stress. If these neurons failed then the energy metabolism for the upstream neurons would be stressed by the additional energetic requirements for neurotransmission and could themselves fail. As required in the Braak staging process, this could cause a domino effect of damage [46] backward up the spinal cord and into the brain stem.

Based upon the above observations, we posit that age/toxin/trauma–related inefficiencies which reduce the ability of the brain's energy metabolism to generate and regulate the required ATP levels may be an important enabling factor in iPD. In support of this proposition, impaired energy metabolism has been revealed in Parkinsonian brains, with decreased glucose utilisation in the area that is most affected in Parkinson's disease – the cortex and basal ganglia [43].

In the wider context of neurodegeneration, studies of oxidative stress in Alzheimer's disease (AD) [20] report a 50 percent reduction of glucose utilisation in the disease brain, and that this change in glucose metabolism *precedes* the clinical manifestation of disease. In a recent editorial, Del Tredici and Braak [45] comment on the mixed AD and PD pathologies with advancing age. Taking the multi-factorial systems view proposed here, we speculate that AD and PD pathologies may share a deterioration in brain energy metabolism with advanced age as a common etiological factor. Thus the weakness in the brain's energy systems may create a common upstream predisposition for a number of neurodegenerative disorders, with other factors (most probably genetic) deciding which particular disease develops and with what specific pathologies.

Energy Based Mathematical Modelling of Parkinson's Disease

Energy metabolism as a modelling framework

As noted previously, the basis of a systems approach in the physical sciences is mathematical modelling of the relevant energy handling components of the system and their intercoupling via an interconnective framework.

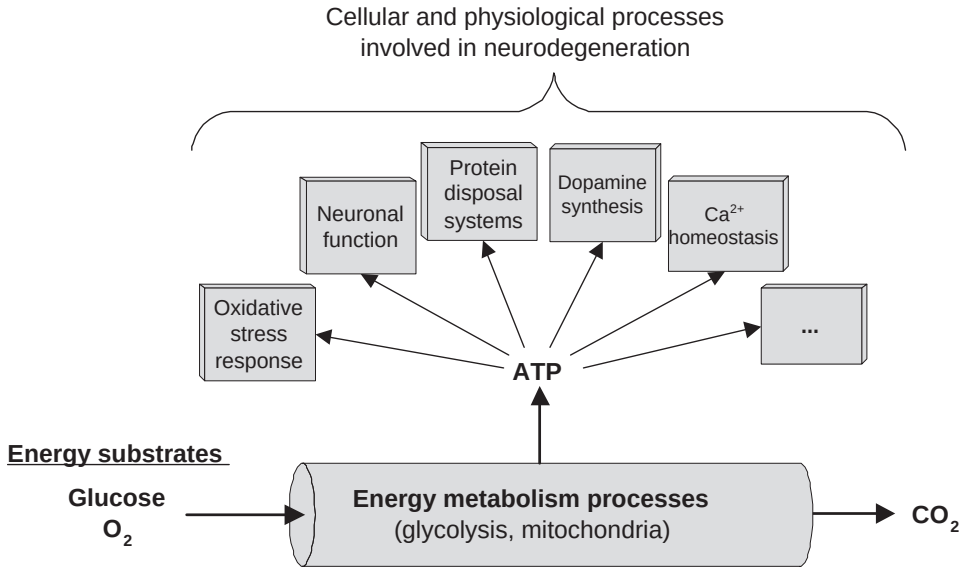

Figure 2: Illustrating the brain energy metabolism as an interconnective structure linking energy sources (glucose and oxygen) and the energy sinks (the ATP consuming cellular functions)

Although the delineation between interconnective components and energy handling components is not as clear–cut in living systems as it is in physical systems, a similar procedure can be adopted. Specifically, we consider the energy metabolism of a living system to be the interconnective framework that links the energy sources (substrates blood glucose and oxygen) and the energy consuming sub–elements which perform the biological functions within a living system. The energy metabolism framework is not purely interconnective, since we incorporate within it the biochemical processes that convert glucose/oxygen into ATP. In the energy hypothesis of iPD this has advantages, since the possible contribution of these conversion mechanisms to disease causation may also be studied.

Since the brain energy metabolism fuels all cellular and physiological functions, it can also be used as a mathematical and computational framework within which to embed models of specific processes thought to be implicated in iPD. In particular, and as shown in Figure 2, once an energy metabolism model is constructed, mathematical models of the various energy consuming biological sub-systems may 'connected' to the energy metabolism framework via the energy flow (as determined by ATP flux) between the metabolism and energy consuming sub–systems. In the case of disease modelling, this approach would allow us to test not only the disease contributions of the energy metabolism, but also potentially the disease contributions of each of the suspected energy consuming sub-systems, plus any combination of sub-systems and metabolism. Thus, by placing the brain energy metabolism at the centre of the modelling exercise, we are able to study the role of changes in energy supply capability in the brain. In this way, an systems approach based on energy metabolism provides

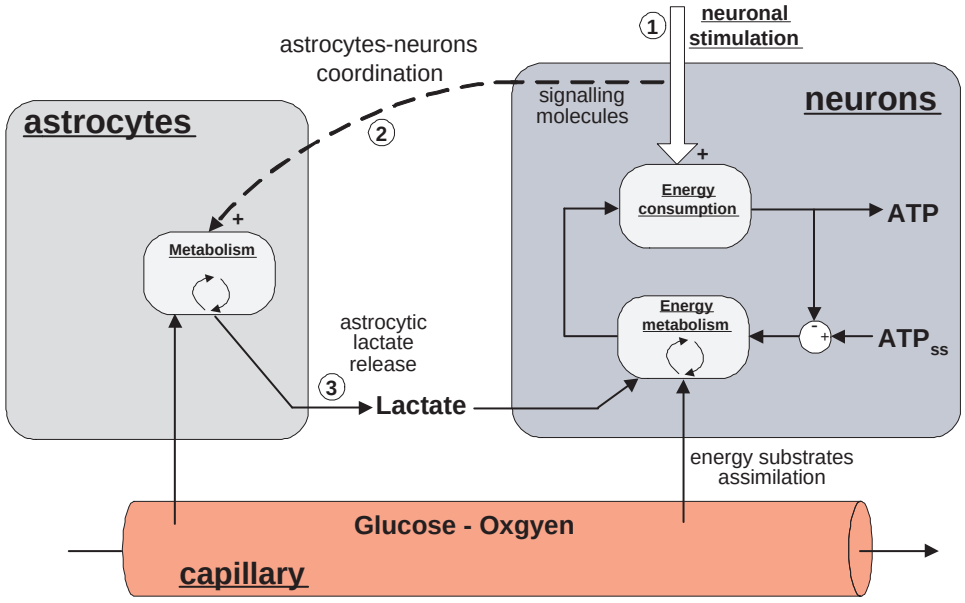

Figure 3: The energy metabolism model, illustrating the principal exchanges between the compartments

both a logical basis in terms of the mathematical modelling, and a scientific motivation in terms of the role of ageing and the reduced efficiency of energy utilisation that comes with age.

A Model of Brain Energy Metabolism

A compartmental approach

Based upon excellent and insightful prior work by Aubert [3], we have developed an integrative mathematical model of brain energy metabolism [14] for *in-silico* studies of PD. The model has been calibrated using time course data from a unique source of *in–vivo* measurements of extracellular neurochemical concentrations [30], and validated against independent *in–vivo* observations of neurochemical dynamics. In this article, we use the model as the basis for an energy–based systems approach to Parkinson's disease. A detail implementation of the model is available from the CellML repository (http://models.cellml.org), here we give only an outline description of the model.

As shown in Figure 3, the modelling framework has a compartmental form, and deals with the energy substrate and neurotransmitter trafficking between four compartments: neurons, astrocytes, brain capillaries and extracellular space. In this figure, the principal flux paths are shown together with the key structural compartments. Thus glucose and oxygen passes to the neuronal and astrocytic compartments, and act as supplies to the glycolytic and mitochondrial processes. With reference to Figure 3, neuronal stimulation (stage 1

in the figure) initiates energy processes in both compartments. Stimulation of astrocytes (stage 2) occurs through neurotransmitter uptake, this in turn coordinates release of lactate from the astrocytes into the extracellular space (stage 3). From there it is taken up by the neurons to help service energy demands of stimulation.

Thus astrocytes, in addition to their well–known structural role, play a biochemical role by clearing neurotransmitters from the extracellular space and supporting energy substrate supply to the neuron [38]. This assistance is relevant to potential energy stress associated with iPD, since it occurs during periods of high excitation [13] and hence high energy requirements. Although there is continuing controversy on the role of astrocytes in brain function (e.g. [38, 19]), we believe that the weight of evidence is that astrocytes are potential players in neurodegeneration, both from the systems viewpoint [13], as well as by other mechanisms [33].

Model description

Viewed in a little more detail, the mathematical model of brain energy metabolism used here [14] and available from the CellML model repository, includes mathematical descriptions of the key glycolytic/mitochondrial energy reactions in both neurons and astrocytes and metabolic fluxes. The quantitative description of the model is given in [14], here we restrict ourselves to a qualitative overview of the model operation. With reference to Figure 4, the model functions as follows:

1. The energy substrates glucose and oxygen pass from the brain blood capillary via the intercellular space to the neuronal and astrocyte compartments.
2. Within both compartments, glucose (GLC) is converted to the intermediate product pyruvate (PYR); in astrocytes an additional process (glycogen accumulation) also allows temporary storage of an energy substrate.
3. The pyruvate is taken up by the mitochondria, either partially (with excess pyruvate converted to lactate (LAC) and transported to the extracellular space), or at times of high ATP demand, completely (when its flux is supplemented from lactate imported from the extracellular space). This shuttle of lactate is supported by astrocytic lactate export to the extracellular space [38].
4. High ATP demand is triggered by neuronal stimulation with concomitant neurotransmitter (in this case glutamate (GLU)) release. The astrocytic uptake of glutamate synchronises the release of lactate into the intercellular space to meet the corresponding increase in neuronal ATP demand. This is the glutamate loop for neuronal astrocyte coordination [21].
5. The quiescent cellular demand for energy in both cell types is represented by the 'Maintenance ATPpase' modules. The main transient demand for ATP is to drive ion channel pumps during external stimulation of neurons.

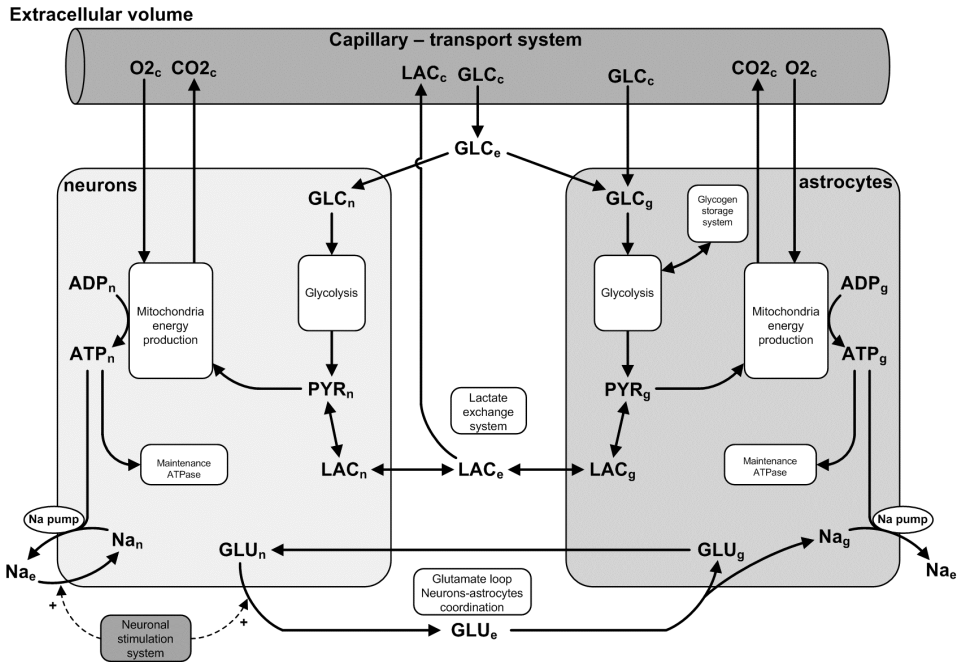

Figure 4: The energy metabolism model, showing the principal exchanges of oxygen, glucose, lactate, glutamate and carbon dioxide between the compartments, together with the key cellular functions.

These are the key biochemical processes represented mathematically in the model (full details of which are given in [14] and associated references). In the energy hypothesis for iPD, important systems elements of the brain energy model are the lactate exchange system and the glutamate loop (neuron-astrocyte coordination) system. These underline the point made elsewhere that astrocytic contributions should not be ignored when researching failures in neuronal systems, e.g. [33]. From a systems viewpoint, astrocytes act as a feedforward mechanism that offers vital supplementation of the neuronal energy metabolism at times of high transient demand for ATP [13] – failure of this supplementation will place the neuron under severe transient energy stress. The word transient is important here, since it emphasizes the importance of dynamics and temporal variations in disease. This point is illustrated in the following two sections.

The Brain Energy Metabolism as a Dynamic Integrated System

When implemented within a computer and validated against *in–vivo* biological data, the mathematical model becomes an *in–silico* tool for investigating brain energy metabolism. The following paragraphs shows how an *in–silico* model validated in this manner [14] can be used to investigate the behaviour of neuronal energy levels over time, including: (i) the integrative action of the control mechanisms which govern neuronal energy levels,

(ii) the 'hidden' variables that cannot be measured *in–vivo* and (iii) the importance of short term transient energy stresses in iPD.

Control and regulation in a healthy brain: the role of astrocytes and the importance of transient information

Parkinson's disease is conventionally viewed as a disease of the neuron. However, this viewpoint changes under the hypothesis that deterioration(due to age, toxins or physical damage) of energy metabolism may be associated with disease susceptibility. Specifically, the control system that supplies of ATP to service neuronal function requires supportive activity during times of rapid need, with astrocytes the most pausible candidates for this assistance. Neurons and astrocytes work as an integrated control system [13] that maintains the availability of neuronal ATP at a constant level during the rapid and frequent demands caused by neuronal stimulation. This has important implications for neuronal function since, without this support, neurons can not maintain all cellular functions during transient stimulation.

In the quiescent state, the neuronal ATP is maintained at homeostatic levels by neuronal glycolysis and mitochondrial regulation. However, during rapid transient energy demands, neuronal glycolysis is unable to respond rapidly enough to match the speed with which neuronal stimulation (and concomitant ATP requirements) occur. To meet this case, it seems that an integrated energy metabolism has evolved [13] whereby fast feedforward from astrocytes can rapidly supply additional energy substrate to meet the transient demand of neuronal stimulation. As shown in Figure 5, the mathematical model can be used to illustrate this integrated control and joint regulation action of the two cell types. The top central panel of the Figure indicates a typical neuronal energy demand in response to transient stimulation – the requirement is for 10% increase from the basal 100%. The left hand curve of neuronal glycolysis show no response to the increased energy demand, however co–activation of the astrocytes (right hand glycolysis response) shows a rapid glycogen–assisted response and a consequent increase in LAC which satisfies the additional transient substrate requirement of the neuronal mitochondria. The integrated action of the two cell types enables the overall neuronal ATP to be maintained at the homeostatic level required to maintain cellular function.

Figure 5 also suggests that the time course behaviour of neuronal activity should be considered in disease pathology. Specifically, when the brain is at its basal level of activity, the astrocytes play no significant role in maintaining the homeostatic ATP levels. However, during stimulation, rapid transient demands are placed on the energy metabolism. As will be shown later, these transients place particular strains upon a metabolic control system that is weakened in some way.

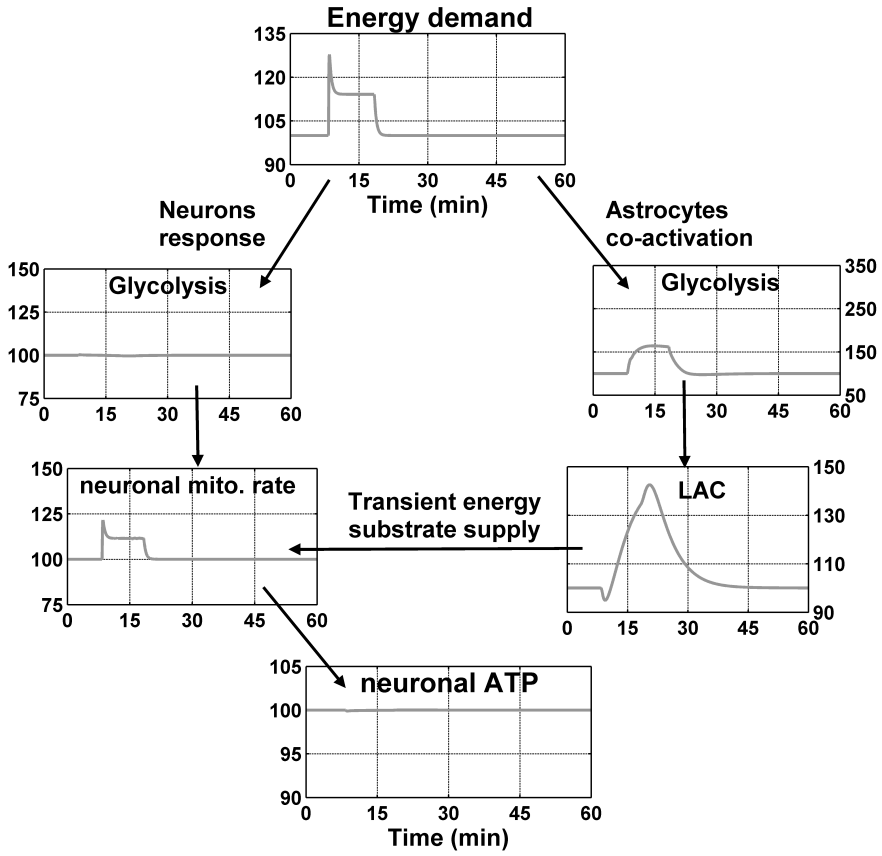

Figure 5: Dynamics and interactions in brain energy metabolism. Upon neuronal activation, astrocytes are co-activated, producing LAC which contributes to the energy supply in neurons. Variables are % of steady-state values

In–silico experiments reveal intra–cellular variables that are unmeasurable *in-vivo*

The (*in–silico*)experiments shown in Figure 5 give insights into the integrated nature of brain metabolism and the strong dependance of neuronal function upon very specific transient time variations. However an *in–silico* model also permits us to trace the variations in informative variables that cannot be measured in living brain tissue or *in-vitro*. These 'hidden' variables are of value in investigating the deeper function of the brain during stimulation. For example, Figure 6 shows the behaviour of intracellular NADH in neurons and astrocytes during a one minute increase in neural activity of 15% above basal (left–most column in Figure 6). NADH can be measured *in–vivo* using fluorescence monitoring and has been used to distinguish the spatial and temporal variations *in–vivo* [22]. The *in–silico* model reproduces observed behaviour in neurons and astrocytes (second column from left in Figure 6), but also the corresponding unmeasurable reaction rates in both neurons and astrocytes (third column from left in Figure 6). These two 'hidden' reaction rates offer a rational explanation for the time course behaviour of the measurable extracellular lactate concentration (right–most column in Figure 6). The figure illustrates the faster activation rate of neurons (compared to astrocytes) which leads to the familiar transient dip in extracellular lactate that occurs before astrocytes are fully activated.

In-Silico Exploration of Compromised Brain Energy Metabolism in iPD

The mathematical model of brain energy metabolism provides an integrative environment for the study of potential contributors to iPD. In this article, we use the *in-silico* implementation of the brain energy metabolism model to demonstrate examples of energy related factors associated primarily with age and lifetime experiences (box B of Figure 1), and how they might open a gateway for PD pathologies (Box C in Figure 1) to develop. In particular, we consider energy–limiting mechanisms associated with life course experiences and the ageing brain, and propose them as upstream factors in idiopathic Parkinson's disease, with the common theme of energy deficiency and/or metabolic impairment. Of importance in these demonstrations is the long time period over which deteriorations develop with ageing, and the ability of an *in–silico* simulation to rapidly evaluate potential disease mechanisms that in real-time could take decades to develop. We consider two aspects of energy regulation: (i) the maintenance of steady homeostatic levels of ATP and (ii) the impact of rapid transient stimulations upon the regulation of ATP levels. First, the steady state regulation case.

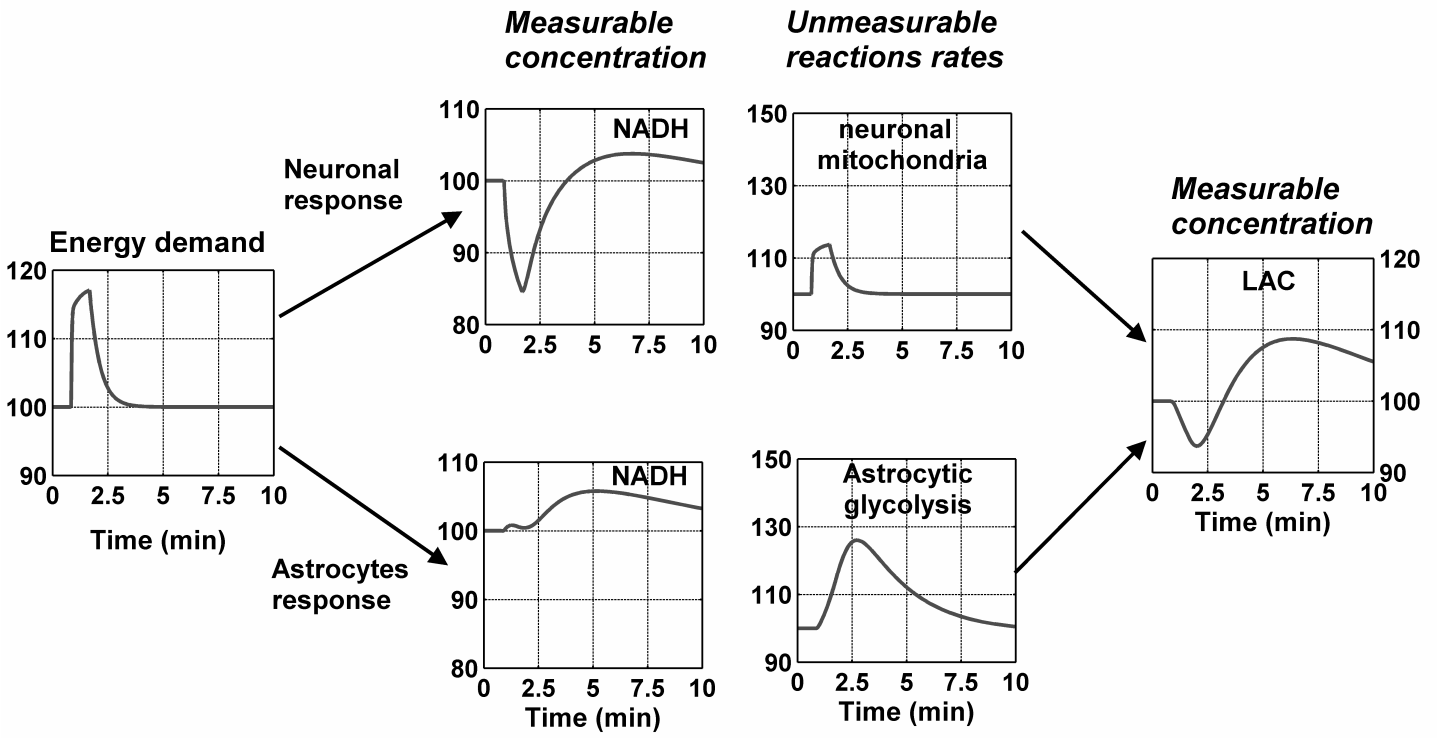

Figure 6: Measurable and 'hidden' (e.g. unmeasurable) variables in the *in–silico* brain energy metabolism. The mitochondrial NADH concentration and extracellular lactate (LAC) can be measured in–vivo. However, the reaction rates that link them cannot and are therefore unobservable during *in–vivo* experiments.

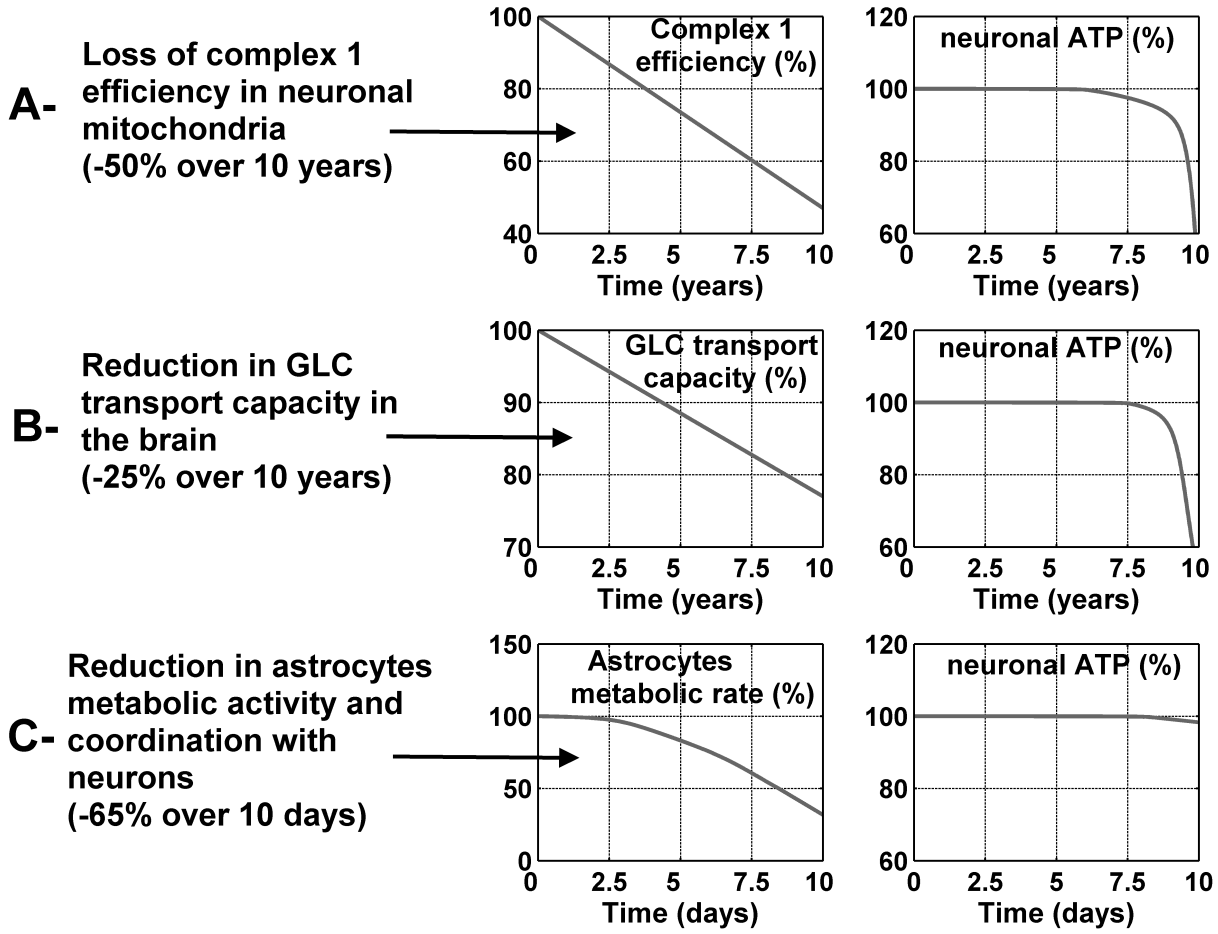

Figure 7: Age and lifestyle related decrease in brain energy metabolism efficiency. The gradual decline in energy metabolism efficiency over time is held in check by the homeostatic control systems for energy metabolism. Eventually the reduction is such that homeostasis can no longer be sustained and ATP availability collapses.

Homeostatic regulation of brain energy

Figure 7 illustrates the influence of gradual reductions in mitochondrial efficiency, glucose uptake and astrocyte activities on steady state neuronal ATP levels. We show deterioration in mitochondrial efficiency because it has been proposed as a factor in neural disfunction in the elderly [42]. In a similar vein, Gibson [20] and others [43] have noted the association of reduced glucose utilisation with neurodegeneration – particularly with reduced uptake in the basal ganglia. A linkage has also been made between head trauma and the subsequent development of Parkinson's disease [6, 41] and in light of this, we suggest an energetic connection between astrocytic/capillary damage induced by head trauma and iPD, whereby severe head trauma can reduce the supportive function of astrocytes and damage the intimate linkage between capillaries, astrocytes and neurons. This would result in reduction of glutamate up-take [1] by astrocytes (itself potentially damaging) and reduce the efficiency of the lactate substrate transport from astrocytes to neurons. Consider these factors in turn:

- **Age–related reduction in mitochondrial efficiency.** Row A of Figure 7 is an *in–silico* simulation of gradual reduction in mitochondrial complex 1 efficiency, with the arbitrary choice of a linear 50% reduction over 10 years. The figure shows the powerful nature of the neuronal regulation mechanisms which are able to maintain ATP constant at the homeostatic level until a certain crucial point is reached (around 6 years in the simulation, or 30% reduction in mitochondrial efficiency). Beyond this point the regulation mechanisms can no longer maintain the biochemical balances required and ATP levels start to collapse. As noted earlier, and using an extrapolation of bottom-up calculations of energy budgets, there is evidence to suggest that the energy turn–over in substantia nigral dopaminergic cells is much higher than elsewhere in the brain. Based upon this observation, we posit that age–related reduction in mitochondrial efficiency would cause a collapse of homeostatic control of energy metabolism to occur first in the dopaminergic neurons of the SN. Neural cells as a whole have a higher basal ATP turn over than other cells, and this may help explain the general susceptibility of the brain to iPD damage.
- **Reduced glucose up–take in the elderly brain.** The centre row of Figure 7 simulates the impact of reduced glucose utilisation by a linear decrease in glucose transport from the capillaries over a 10 year period. Again, the metabolic regulatory mechanisms hold cellular ATP at the required levels until a critical minimum level of glucose utilisation is reached at around 7.5 years, when homeostatic control collapses. In a comprehensive cell model, the programmed cell death sequence would have been triggered before the total collapse in ATP levels shown here. The previous comments concerning the particular susceptibility of SN neurons also applies in this example. Although our simulation does not reproduce the increased striatal lactate observed in [43].

- **Head trauma and brain energy metabolism.** Head trauma might feasibly impair the brain energy metabolism through the dependance of neurons on energy substrates from astrocytic cells. The implication of this impairment is shown *in–silico* in the rest condition in row C of Figure 7, where the damage caused by the impact of a linear reduction in astrocytic contributions over the arbitrary period of 10 days is shown. This shorter timescale was chosen to show that modelling can equally well describe long-term or medium term periods. Notice, that although the metabolic activity of the astrocytes is reduced by 65%, the homeostatic level of neural ATP is maintained, with an almost negligible decrease in neuronal energy, with a visible effect only if astrocytic activity is impaired by more than 50%. As was mentioned in more detailed analysis in Cloutier et al. (2009), this model shows that in resting conditions neurons rely on glucose to maintain 92% of the flux to mitochondria. What we see here is thus resting neurons operating their energy regulation practically 'on their own'. Again, care should be taken in analyzing these results: (i)– neurons do not experience resting conditions for such a long period; and (ii)– astrocytes provide more than metabolic support (in the form of LAC) to neurons. As we will see later, this particular case will be extremely important in understanding how transient conditions are critical when studying brain energy regulation and neurodegeneration.

Note that in a comprehensive model that captured all cellular functions - such as oxidative damage, apoptosis or autophagy - cell death would most probably be triggered somewhere before the homeostatic collapses shown in Figure 7. At this preliminary stage of its development, the mathematical modelling used for the *in–silico* simulation can describe the loss in energy homeostasis, but not the physiological outcome of such a loss. However, the model still gives *qualitative* and *quantitative* insights into the robustness of energy homeostasis in neurons with regard to changes in parameters associated with the category B in Figure 1. At the qualitative level, the modelling shows that energy regulation is not a linear phenomenon and sharp changes can be observed after long periods of homeostasis. The nonlinear behaviour of biological systems is obviously a well known phenomenon to experimentalists (e.g. dose-response curves, Michaelis-Menten kinetics...). Our background in dynamical systems analysis leads us to believe that nonlinear behaviour will be important in understanding disease development. Regarding the quantitative aspects, estimates provided here could be useful for further validation at the *in–vitro* or *in–vivo* level. Quantifying experimental conditions and results in biology will be of critical importance. Here, for example, our modelling experiments show that an *in–vitro* experiment with a treatment that induces a decrease in complex I efficiency would potentially yield different results just because of a small change in the treatment's strength or duration.

Regulation of neural ATP during transient stimulation

As was mentioned earlier, the mathematical model can be used to investigate the dynamics of brain energy metabolism – something that is not generally possible at the experimental level. For example, it is doubtful that *in–vitro* experiments correctly reproduce the *in–vivo* dynamics of neuronal stimulation. The model used here, although an *in–silico* abstraction, was calibrated to reproduce *in–vivo* data obtained during neuronal stimulation [30]. We therefore believe that it is justifiable to use the model to study cerebral energy failures in transient conditions associated with neuronal stimulation. In this spirit, we revisit the three case studies of Figure 7, but this time adding the stimulation associated with regular cerebral spiking activity (such as might be associated with SN pace-making) over the time course of the simulated experiments. In this situation, we get a much more realistic picture of conditions in the living brain, where various stimuli continuously increase cerebral activity. The cases A–B in Figure 8, (relating to impairments in mitochondrial activity and GLC transport) now show how the active brain can experience transient reductions in energy levels a long time before the steady-state homeostasis is seriously compromised. We thus get a much more detailed picture of how and when neurons might enter a 'danger zone' where low ATP could lead to irreversible damage.

Case C in Figure 8 now shows the importance of astrocytes for the transition between resting and active conditions in neurons. As soon as the decrease in astrocytic contribution is induced, we observe downward spikes in available neuronal ATP during stimulation. In normal conditions this is not observed as astrocytes only provide lactate for a fast activation of neuronal mitochondria. Here we see that the gradual loss of this key systems property (coordination between neurons and astrocytes) leads to a surprisingly quick loss of dynamic ATP regulation in neurons. Although the steady-state level of ATP is maintained, the transient conditions shows ATP levels dropping by more than 10% even in the early stages of astrocytic damage.

This simulation of transient conditions thus suggests that impaired energy levels in neurons is a phenomenon that will not necessarily be captured in steady-state measurements. The transient transitions between resting to active state also have to be considered in order to get a complete picture of possible energetic failures in the brain. From these simulation results we infer that the cellular damage caused during these deep transient ATP deficits could accumulate over time, opening opportunities for cellular pathogenic mechanisms associated with PD and eventually leading to cell death. These are only *in–silico* results, but they strongly suggest the idea of considering the dynamic nature of the cerebral tissue in further studies on the role of energy in neurodegeneration.

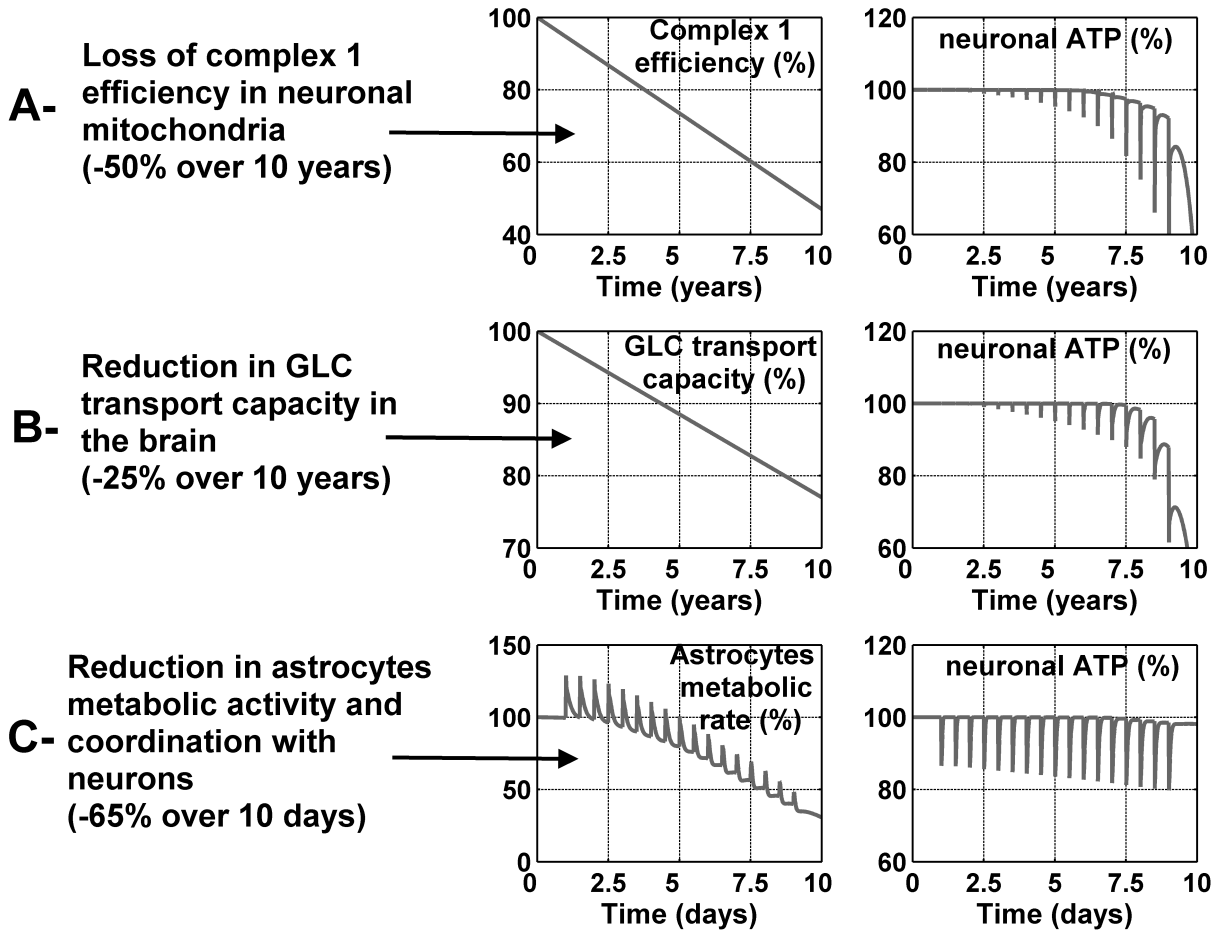

Figure 8: The impact of transient energy demands with compromised brain energy metabolism efficiency. Neural stimulation causes temporary reduction (dips) in available ATP – the dips become greater as the level of energy metabolism effectiveness decreases.

Conclusions

Integrative studies of for a multifactorial disease

A framework for an integrative systems approach to iPD has been designed to enable the multifactorial nature of the disease to be explored. In this position paper, the model framework's potential for the *in-silico* study for disease mechanisms has been demonstrated only as it regards the possible etiological role of compromised energy metabolism. Even at this level, the need to integrated multiple factors involving both astrocytes and neurons has been shown. But this is only a preliminary demonstration of the systems approach's potential – the brain energy metabolism model is a framework upon which to build a comprehensive modular *in-silico* model that allows the addition of any number of suspected etiopathological factors. Sub-models of factors thought to be implicated in Parkinson's disease can added one-by-one to the framework using the energy dependence of the cellular processes as the connective mechanism. Our immediate next step in this direction will be to add sub–models of alphasynuclein and oxidative stress/response to the model framework. This will allow a multifactorial investigation of interactions between reactive oxidative species, alphasynuclein and energy metabolism.

The importance of dynamic and time dependent behaviour

An important feature of the integrated framework for *in–silico* study of iPD, is the ability of this systems approach to show how disease factors behave over time and interact dynamically. Specifically, it is likely that the development of Parkinson's disease involves both long term temporal changes (e.g. ageing) and the cumulative impact of many rapid transient changes (e.g neuronal stimulation) sustained over a long period. In this context, we have seen in Figure 7 and 8, that transient energy demands will stress the brain energy metabolism well before basal energy levels are compromised. Thus we suggest that iPD is not initially associated with deterioration in the basal energy levels, since as shown in Figure 7, this is maintained by strong steady state homeostatic regulation mechanisms until eventual catastrophic collapse. Instead it may be the slow cumulative affect of very many repeated transient energy deficits experienced over a sustained period that opens a gateway for cellular damage to develop very slowly and over many years – just as is observed in Parkinson's disease – and well before the critical point for homeostatic collapse is reached.

Etiology of PD

As a subtext to this article, we have introduced the idea that a compromised brain energy metabolism may be an etiological trigger for a variety of PD inducing pathologies to proceed. In a healthy cell, imbalances in cellular activities or flaws in biological processes are held in check by robust cellular energy regulation systems

that are capable of supporting all cellular functions. However, when cellular energy levels are reduced it is possible that cellular damage (such as proteinaceous accumulations) can proceed on an incremental basis and to an irreversible point. The same idea can be applied at the other end of the Parkinsonian spectrum of familial Parkinson's disease. Here severe genetic mutations are sufficiently serious on their own to create a specific way for pathological mechanisms to proceed. However, moderate genetic abnormalities also play a role in the energy hypothesis for iPD, thus suggesting a spectrum of etiological conditions with the wholly familial and wholly idopathic conditions at either extreme. This idea of a continuum of etiological factors is coherent with the wide spectrum of symptoms that come under the description of Parkinson's disease.

Energy energy systems approach to neurodegeneration

The modelling of brain energy metabolism discussed here has been presented in the context of an age-related view idopathic Parkinson's disease. However, we believe that it can also offer a mathematical modelling, analytical and computational framework for *in–silico* study of other age–related neurodegenerative diseases, such as Alzheimer's. The factors that specialise the modelling framework for a particular neurodegenerative disease are the cellular sub-systems that we might install within it. For example, installing a model of the beta amyloid metabolism within the model structure would orientate it toward an *in–silico* tool for Alzheimer's studies, in the same way that the incorporation of an alpha synuclein metabolism model orients toward Parkinson's disease.

Assumptions and predictions

The two main propositions in this position paper are (a) that a mathematical model of brain energy metabolism can be used as the basis for a systems approach to Parkinson's disease and potentially other forms of neurodegeneration, and (b) that energy deficiency may be a key player in creating a predisposition to the cellular damage associated with Parkinson's disease. With regard to the role of energy deficiency, an underpinning assumption in this is that there is a hierarchy of energy requirements in living cells, with SN dopaminergic neurons being the most demanding by up to two orders of magnitude. This statement is based upon an extrapolation of bottom-up calculations of brain energy budgets for glutaminergic cells [2] to SN dopaminergic neurons. The extrapolation was made by scaling the estimates of the number of synapses and axonal lengths of SN neurons [34] in relation to other neurons, using figures for the rat brain. Experimental work that tests this extrapolation would strengthen the energy approach outlined here.

As mentioned earlier, mathematical models and *in–silico* studies can offer guidance for discovery in practical biology. In this spirit, the *in–silico* results shown here offer a number of useful predictions that are testable *in–vivo* and *in–vitro*. They include:

1. Energy deficits in neurons will lead a cell to preferentially neglect certain maintenance activities which, in turn will allow PD damage to occur. As a specific example, we expect that an energy deficit will cause the neglect of protein recycling in cells. If alphasynuclein (a main component of Lewy Bodies) is over–expressed, then this would lead to an accumulation of alphasynuclein in the cytosol.
2. Regular and frequent transient deficits in energy associated with stimulation will have a cumulative damaging impact upon a cell. This cumulative damage will start to grow long before the reductions shown in Figures 7 and 8 lead to collapse in neuronal energy homeostasis.
3. Head trauma is assumed to cause damage to the capilliary/astrocytic connective structure in the brain, initiating a reduction in astrocytic energy support to neurons during stimulation.

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