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Stroke on a cellular level: The butterfly effect.

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Abstract
Background: There is a comprehensive research focusing on the vascular events and the pathophysiology of stroke. Less focus has been on the events in the individual cells when their metabolism is compromised. The cells try to repair damaged energy producing structures in the mitochondria with tools that themselves require energy to function properly.
Results: The analysis describes a biosimulation model, where the ATP-producing enzymes (the energy cogwheel) and the enzymes repairing the cogwheel are taken as two variables. The interplay between these two variables reveals an extremely sharp borderline between cell death and cell survival after a damage. Its value depends on the size of the damage, cell metabolism, and the dynamics of the damage.
Conclusion: The results point out several procedures that could minimize the damages at a single cell level. Further analysis is needed to combine the vascular research with the single cell findings.
Key words: Biosimulation, stroke, cell damage, cell energetics, ATP control, butterfly effect.

Introduction

Stroke is a cerebral event caused by insufficient perfusion of parts of the brain, like vessel thrombosis, bleeding, or arterial spasms. Common for the events is that part of the brain cells become hypoxic and risk serious damages, if the blood flow is not rapidly restored.

Therefore, the study of stroke is focusing on the vascular damages leading to nerve cell injury and the more general effect on the tissues (Crabbe 2007, Madia *et al.* 2024, Rehman *et al.* 2024, Salaudeen *et al.* 2024).

Most brain metabolism goes to maintain the concentration gradients of sodium, potassium, and calcium ions, both during rest and during brain activity. The nerve cells of the gray matter are unmyelinated, thin with a high surface-volume ratio, and the many synapses require large amounts of energy. Consequently, the gray matter is very sensitive to energy shortage.

Damages after stroke are often assumed to be due to insufficient restoration of the blood flow.

The paper presents another effect of stroke on a cellular level, where small differences in timing and severity of the stroke and in the cellular activity may have fundamental influence on the outcome. It can be compared to the butterfly effect in nonlinear dynamics (Lorenz, 1972: Does the flap of a butterfly's wings in Brazil set of a tornado in Texas?).

The analysis presents the results of a relatively brief closure of the blood flow to a part of the brain. With an ischemia of this duration, many patients survive the ischemia, but they may develop necrosis in parts of the brain.

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The energy cogwheel

The energy flow in the cell from nutrient breakdown to energy usage follows a series of steps that are interwoven like cogwheels.

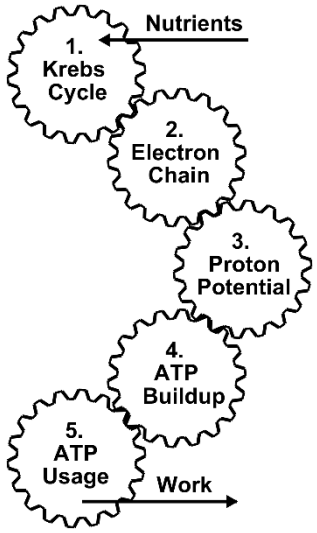

Fig. 1. The energy cogwheel.

1. Krebs cycle: Nutrients are broken down in the cytoplasm, pass into the mitochondria, and enter the TCA cycle as acetyl-CoA. Here they are broken down to CO_2 and H_2O. The energy is released as NADH and $FADH_2$ that moves to the electron chain in the mitochondrial membrane.

2. Electron chain: Electrons are released and transported via large enzymatic complexes, and combined with molecular oxygen, O_2, to give water. It is here oxygen is needed to extract the energy. The intermediate species, free radicals with an unpaired electron, are very reactive and destructive, almost like radioactive radiation.

3. Proton potential: The electron chain uses the liberated energy to pump protons, H^+, out of the mitochondrion. This creates a large membrane potential difference ≈150 mV. The potential varies with nutrient and oxygen supply, and energy utilization. The electric potential difference is used as a battery to build ATP.

4. ATP buildup: The mitochondrion has an extra, modified proton pump, ATP synthase, where protons can flow inwards due to the potential gradient. Inside, there is a propel-like mechanism that can convert the electric energy and catalyze the reaction ADP+P+energy → ATP. (Nath and Jain, 2000) Thereby, internal ADP is converted to ATP that flows out through an ATP/ADP transporter in the mitochondrial membrane.

5. ATP usage: The synthesized ATP is transported out to the cell cytoplasm and diffuses to the organelles that require energy (*e.g.,* muscles, glands, and particularly pumps). Her ATP is reduced to ADP that diffuses back to the mitochondrion for reloading of energy.

The cogwheel system: The 5 steps are tightly bound together. In steady state, there is a synchronous rotation of the cogwheels with a flow from top to bottom. Three cases may be highlighted.

One is an increased level of nutrients. In a normal chemical reaction, this would lead to an increased reaction rate through all the steps. But the speed of the last cogwheel must follow the energy utilization precisely, so if the workload is small, all the cogwheels will be in a "push" position. Especially in the electron chain this can create reactive oxygen species (ROS) that can damage the cell.

Another case is a heavy workload on the cell, where the ATP production is limited by insufficient nutrition. This gives a "pull" situation, where the energy usage depends on the ATP production. The cell may produce a limited amount of ATP by glycolysis, but this is rarely sufficient (Arunachalam *et al*. 2023).

The third case is relevant for stroke. During hypoxia, the complex setup of the interwoven cogwheels may be disturbed or even destroyed. And the malfunction of just one cogwheel may stop the whole chain. The result is that the ATP production is reduced until the cogwheels are repaired or replaced. This takes time, during which the shortage of ATP adds to the damages. To this comes that the repair itself is ATP-dependent.

Model layout

The events taking place during ischemia are numerous, and it is not possible to point out a single factor that causes the damages. Many complex models have been presented, *e.g*., Kumer and Jafri (2022), but here a simple biosimulation model is used.

The design of the model is based upon the following assumptions:

- The function of the cogwheel is mostly ATP-independent. In case of a brief ischemia, it is more or less intact, but the ischemia destroys part of the more sensitive organelles, enzymes etc., and pump failure may change the intracellular ion milieu considerably.
- In the hours after the ischemia, the breakdown of the metabolic enzymes may be faster than their reproduction, so the amount decreases. At the same time, the damaged structures are repaired, which leads to an increase in the production capacity (Liu et al. 2018, Prashar et al. 2024). This balance between breakdown and renewal is the backbone of the model.
- Both the production of the metabolic enzymes and the reparation of the damaged structures costs energy. As the amount of metabolic enzymes decreases, the cell metabolism also decreases. This leads to a slower restoration and sharpens the acute borderline between cell death and recovery.

A thorough description of the model and the normalization of the variables is given in the appendix. Here only the normalized model is presented.

To simplify, the energy-rich substances (ATP, PCr etc.) are treated as one: ATP, and the energy-low as one: ADP. With constant nutrition level, the ATP production of the cell depends on the number of mitochondrial sites, E, (cogwheel sets) and their rotation speed. While the number of active sites may change after the hypoxia, the rotation speed depends mainly on the energy use D (cogwheel 5).

As described in the appendix, the balance between cogwheel 4 and 5 determines the A=ATP/ADP ratio that is used as a measure of the cell's energy state, so with normalized values and ε as a constant:

$$A = \frac{ATP}{ADP} = \frac{E \cdot (1-\varepsilon+\varepsilon \cdot D)}{D} \quad (1)$$

The breakdown and repair of E is described by the equation:

$$\frac{dE}{dt} = k_\alpha \cdot (M(A) \cdot Q - E), \quad (2)$$

where Q is the amount of enzymes etc. that repair the cogwheel, k_α is a rate constant, and M(A) is a function that describes the energy dependence of the Q-activity.

The Q describes the control system that keeps the cogwheel sites and the mitochondria functioning according to:

$$\frac{dQ}{dt} = k_\beta \cdot (M(A) \cdot F(A) - Q), \quad (3)$$

where F(A) is a negative feedback function, and k_α is a rate constant.

$$M(A) = \frac{(1+K^n) \cdot A^n}{K^n + A^n}, \quad (4)$$

$$F(A) = \frac{1+K^n}{K^n + A^n}, \quad (5)$$

M(A) is a high order saturation function, which is 1, for A=1. F(A) is taken to use the same order and ensures that a high A gives a small feedback and small A, a high feedback. Other parameters are K=0.7, n=7, k_α=0.5 h^{-1} and k_β=0.25 h^{-1}. The constants represent a large spectrum of time scales and could be larger or smaller (Yakes and Houten, 1997), but with the simple system of two differential equations, it just corresponds to a scaling of time. The variable D is assumed not to be influenced by the small energy demand for the production of E and Q, but it may vary with time due to other metabolic events in the cell.

Results.

Part I: D=1.

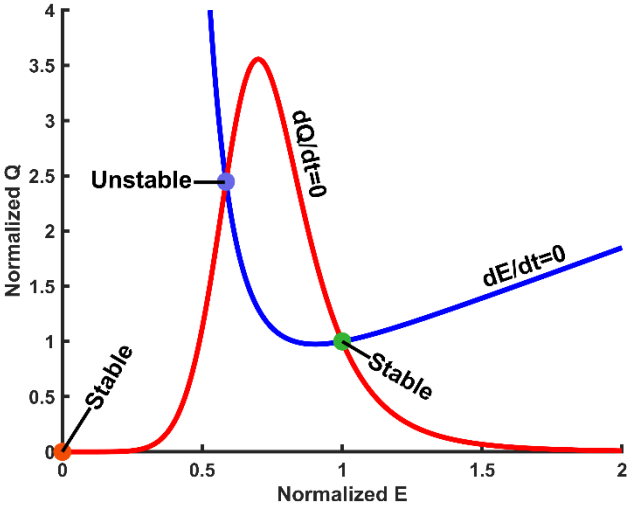

Fig. 2. The nullclines for the two variables with the 3 related fixpoints.

A common way to analyze a nonlinear system is to plot the nullclines, i.e., the curves, where the derivative of one of the variables is zero. Fig. 2

shows the relation between E and Q for dE/dt=0 (eq. 2, blue) and that for dQ/dt=0 (eq. 3, red).

The two curves have 3 intersections. The first (1,1), green, is the stable, basic state, where the cell is functioning normally. The second (0,0), red, is also stable, but here the cell is irreversibly destroyed.

The third (0.58, 2.44), blue, is special. It is a possible, steady solution, and the cell could, in principle, stay there forever. But any small perturbation will grow in time and move the system away from the unstable point to end up at one of the two stable points.

The system starts at the green, stable point, and after 1h, the Q is suddenly reduced to a lower value, whereafter the system continues on its own. Fig. 3a shows E as a function of time for a reduction in Q going from 1.0 (Q=0, darkest blue) to 0.05 (Q=0.05, darkest red) in steps of 0.05.

In the recordings with a high reduction (blue), the value of E goes down to zero within a few hours, while those with smaller reduction (green to red) returns to the basic state after some oscillations.

The division between the two fates is sharp, which is demonstrated with the two red curves in between. For the upper curve, the reduction in Q is 0.77987727139093593, while it in the lower curve is 0.77987727139093594, a difference of 1.28×10^{-17}.

The distinction between the two fates is more than razor sharp. Taking a razor blade of 0.2 mm, the found difference corresponds to an edge thickness of a factor 2.5×10^{-21}. An iron atom has a radius of 126 pm or 1.26×10^{-10} m. so the threshold is some 50 billion times smaller than a single ion atom. In this context, the flap of a butterfly's wings feels like a tornado.

Fig. 3b shows the fate of Q under the same circumstances. Here Q rapidly moves from its reduced value back to the basic value or even above this. Again, the blue curves end up at Q=0, and the green to red curves at Q=1.

More surprising is the behaviour of the two borderline cases. Again, they follow each other in the beginning and reach up close to the Q-value, 2.44, of the unstable fixpoint, before they both decline. One goes towards zero. The other ends at Q=1 after some oscillations.

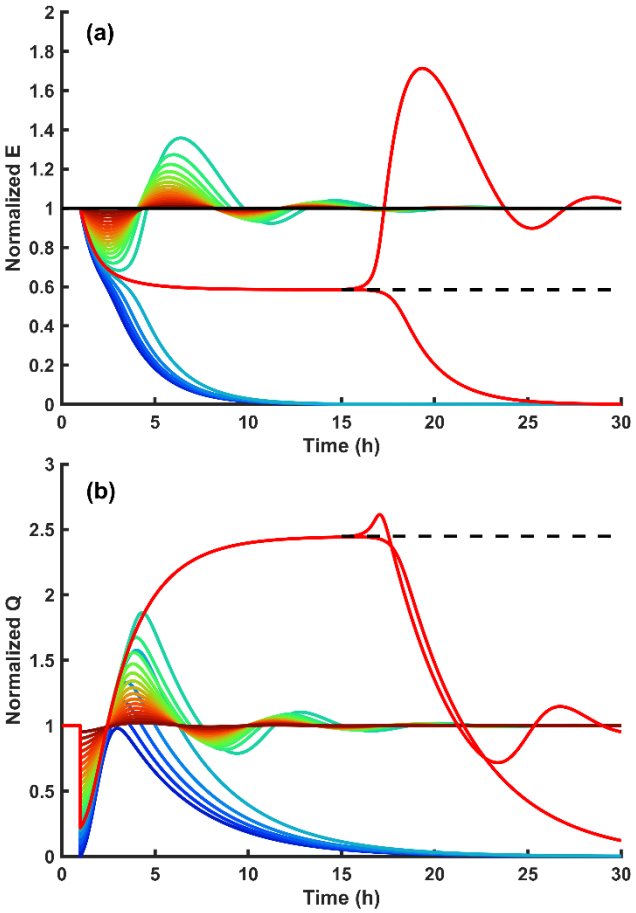

Fig. 3 (a): The value of E as a function of time after reduction of Q from 1 (darkest blue) to 0,05 (darkest red) in steps of 0.05. The broken, black line corresponds to the E-value at the unstable fixpoint (0.58,2.44). (b): The value of Q as a function of time like Fig.3a. The broken, black line shows the Q-value at the unstable fixpoint (0.58,2.44).

Fig. 4 shows a phase diagram of Fig.3a and Fig.3b, where Q is plotted as a function of E. The colour spectrum is clear with a distinct difference between stable and unstable cases.

The borderline cases go from the almost common value at E=1 up to the unstable blue fixpoint, where it divides in an unstable case going down left to zero and a stable case going to the right and ending at the green, stable fixpoint. The broken, black line from E=1 to 2, marks the continuation of the border between initial values of (E, Q) that leads to the red zero fixpoint and those leading to the green, basic fixpoint. The continuation of this is the red line going to the unstable fixpoint and continuing upwards following the blue nullcline.

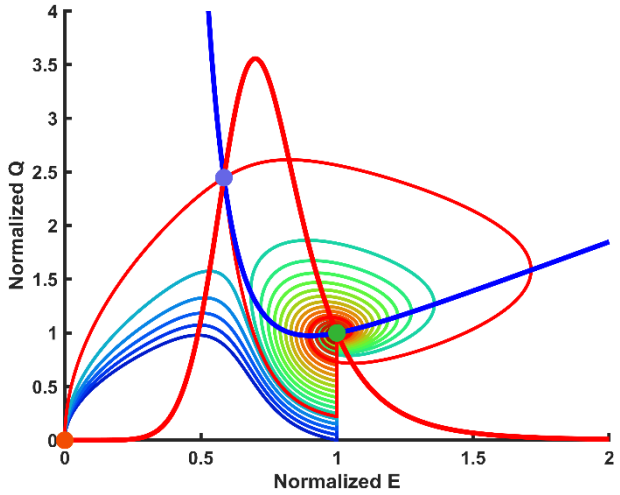

Fig. 4. Phase diagram with Q as a function of E from Fig.3a and 3b. For details see text.

In this way, the Q-E plane is divided into two regions: One, the lower left, leading to the red, zero fixpoint, and another, the higher right, leading to the green, stable fixpoint. Hence, the precise type of mitochondrial damage is crucial for the chances of cell survival.

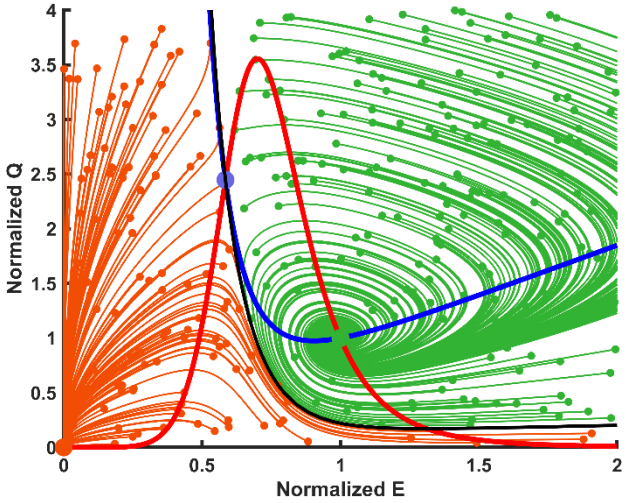

Fig. 5. Separation of the plane. A random set of initial values (points) followed by lines either to the green or the red point.

The division is shown in Fig. 5. The system is started up with a random value of the initial E and Q. The values leading to the red point are depicted as red points with a line that shows the movement of the phase plot for these values. Similarly for the green curves that go to the green point.

Notice that solutions starting near each other continues to be near, and a solution starting on top of another line, follows it to the end. And none of the curves cross the black borderline.

Part II: D ≠ 1

When D differs from D_0, the functions M(A) and F(A) depend in a different way on E, so the phase plot corresponding to Fig. 2 changes as shown in Fig. 6.

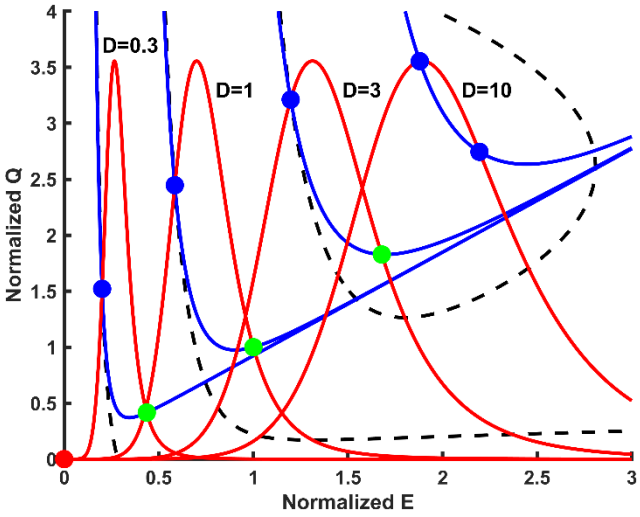

Fig. 6. Phase diagram with different values of D as indicated on the figure. Broken lines show the corresponding border lines.

Generally, the curves move to the right and broaden with increasing D, but at the same time the curve dE/dt=0 moves upwards, so two of the steady state solutions move upwards, while the last point (E=0, Q=0), remains constant. For large values of D, the two curves do not intersect, so the only steady solution is (0,0). It is also seen that for D=10, both the points above zero are unstable, so also here the only solution is (0,0).

The broken black curves show the sharp borderline between solutions leading to the red or green points. For D=0.3, the area leading to (0,0) is small, and even with Q=0, the cell can go back to the stable point, if E>0.285. The case for D=1 has been shown before. A large part of the plane is safe, but E must not be too small (>0.53), and a Q>0.171 is in all cases necessary to go back to (1,1).

For D=3, the borderline is particular. The stable area covers a limited part of the phase plot. The remainder is unstable. This means that a cell operating with D=3 is somewhat stressed and vulnerable. A small perturbation may bring the system outside the stable area, whereafter it will move to (0,0). For D=10, there is only one

stable point (0,0), and no borderline. From anywhere in the phase plane, the system ends up at (0,0).

So, if the system is started close to a stable solution, it will move to that stable solution and stay there. If it is started at a state corresponding to the borderlines or an unstable solution, any small disturbance will move the system away from this state to a stable solution, so these states represent unstable solutions.

The path towards (0,0) is special. The cell is destroying more of the enzymes, E, than can be rebuild by the enzymes Q, so both E and Q go down. As the reason is the constant, too large drag on the metabolism that prevents the enzymes to be rebuild fast enough, the cell may – in some cases – try to save itself by reducing the energy consumption and the damages that follow the enlarged metabolism.

The effect of reduced energy consumption is demonstrated on Fig.7a. Here the cell is started with D=1, but after 4 h, D is reduced to 0.3. The thin, black line shows the borderline for D=0.3. The upper curves, red to green, move in the beginning back to the steady point, but thereafter they move to the stable point for D=0.3. The lower curves, blue to green, move differently. The two first blue curves and up at (0,0). The explanation can be seen from the black points that correspond to time 5 h, where D goes from 1 to 0.3. The first two points show that the curves have passed the borderline, before D is reduced. For the following curves, D is 0.3 before the borderline is passed, so they return, first towards (1,1) then to the stable point corresponding to D=0.3.

The third curve moves for 1-2 hours along the borderline before it moves back and ends up saved. Again, this shows the importance of the borderline. If the system crosses the line, it ends up at zero, but if it does not, it can end up saved, even if it has passed the borderline for D=1.

Fig.7b shows how the red and green curves have time to move back to the steady point, E=1, before D is reduced, and they move down to the steady point for D=0.3. Hence, the sequelae of a stroke can to some extent be reduced by reducing the energy drain of the cell. After the acute phase, the system can slowly be brought back to normal state with limited damages.

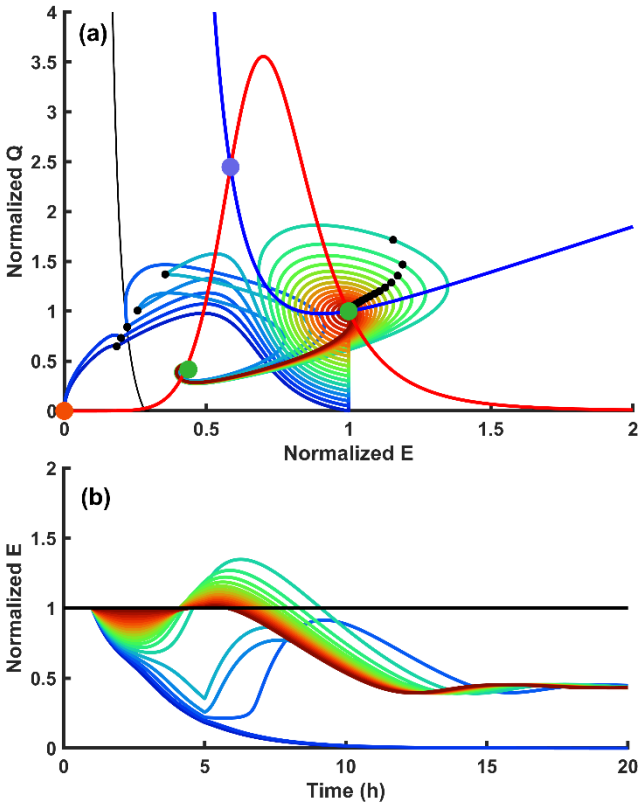

Fig. 7a and 7b. The system starts with D=1 and the same reduction in Q as before, but D is reduced to 0.3 a 5 h.

So, the cell responds to an increase in the energy demand by increasing E and Q. This ensures a relatively constant ATP concentration, so other processes than the energy demanding process can run. The cell can sustain a rather large energy demand, but it becomes increasingly vulnerable as the demand increases. If the demand is too large, the cell dies.

Part III: Varying D

Constant change rate: First the effect of a step/ramp change in D is considered. In the present case, the drain is changed at a constant rate from the initial value of 1 to 3 and held there. The rate is characterized by the time it takes to go from D=1 to 3.

This is shown on Fig. 8. Both the initial and the final state are stable, but if the change is too fast, the cell cannot adjust E and Q in time to prevent cell death. In the figure, the slope is going from 0.01 h^{-1} (dark red) to 0.2 h^{-1} (blue) in steps of 0.01 h^{-1}, corresponding to an increase in D from 1 to 3 in 100 or 5 h, respectively.

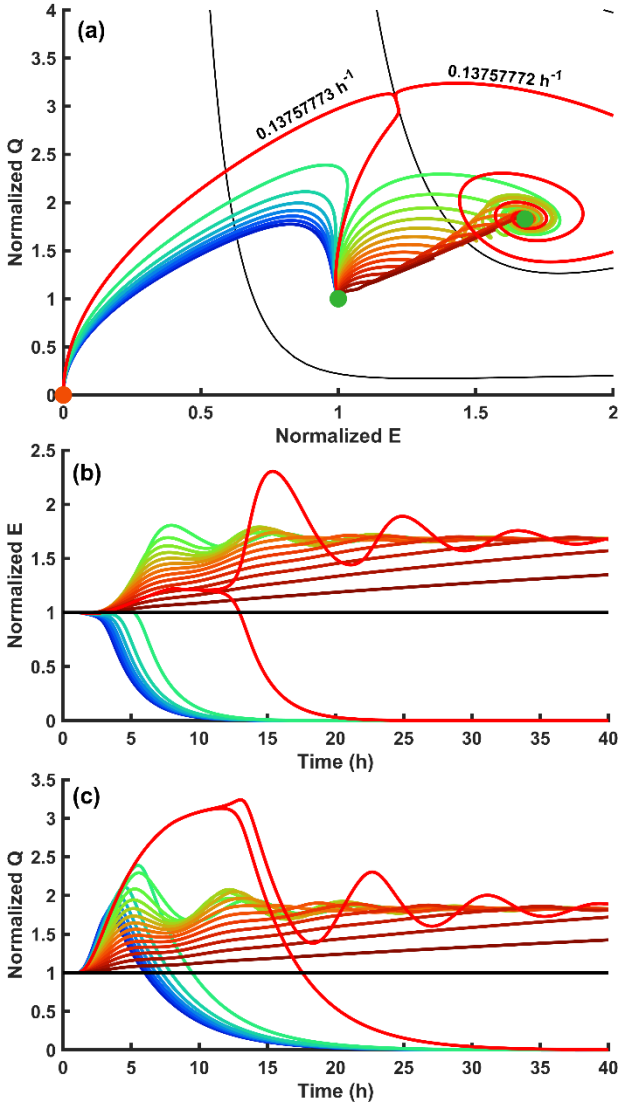

Fig. 8. The system is starting in the basic state. After 1 h D is increased linearly from 1 to 3. The colours are now displaying the change rate from 0.2 h^{-1} (blue) to 0.01 h^{-1} (dark red).

If the change is slow, the stable state is reached, eventually after some damped oscillations. If the change is very slow, the path will be an almost straight line between the two points, but even with a duration of 100 hours, there is some irregularities due to the oscillatory nature of the system.

The edge between the two states is again more than razor sharp. Here the two red curves are drawn with a rate of 0.13757772 h^{-1} or 0.13757773 h^{-1} as indicated on the figure. The limiting duration is near 7.27 hours, and again the limit is very acute. The limit depends on both the initial and the final drain value.

Note that there is no loss due to ischemia. The problem is instead to adjust the values of E and Q to the increasing burden.

Diurnal variations: When a dynamic system is forced by an outer oscillation, complex resonant or chaotic patterns can occur, if the system has some inborn tendency to oscillate with a frequency in the same order of magnitude as the forcing frequency. And as demonstrated earlier, the system has a tendency to show damped oscillations with a period of 5-10 hours, which 25-50 % of the diurnal period.

Diurnal variations are present both in the general metabolic rate and in the concentration of most hormones, and the same is the case for the metabolic drain on the nerve cells.

With the acute borderline between necrosis and survival, even small variations in the energy drain are important. To this adds the marked sensitivity for disturbances, so it must be expected that the threshold for necrosis shows considerable diurnal variations.

This is demonstrated in Fig.9. The system is at a "steady state" driven by a sinusoidal 24 h variation in D from 0.5 to 1.5. The resulting relation between E and Q is shown in all phase plots as the irregular closed, dark red curve in the middle. The black line demonstrated the phase plot of D.

At a given clock time, 0, 6, 12 or 18 h, the value of Q is suddenly reduced in the same fashion ad in Fig. 4. It is seen that the outcome is strongly dependent on the time of day, or rather the value and behavior of D.

In the first case (00:00), D is low so in most cases, the cell will survive, particularly those that manage to get back to the steady curve, before D is increased too much. In the second case (06:00), the heavy load of the large D and even more the increasing D gives a limited survival. In the third case (12:00), D is at its highest, but decreasing from now on, so the survival is higher. Finally, in the fourth case (18:00), the stroke takes place at a time, where D is medium, and where it is rapidly decreasing, so all cases are saved.

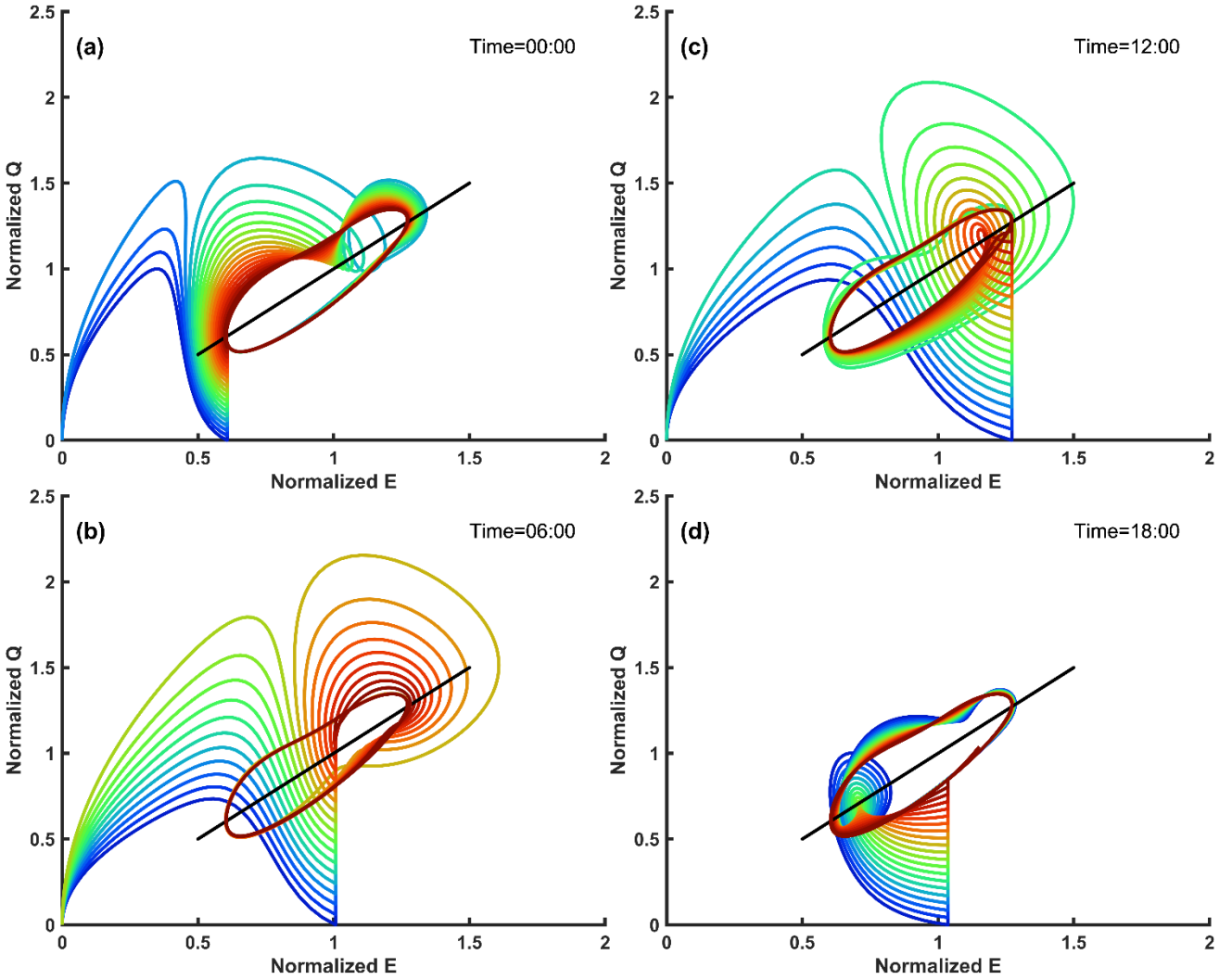

Fig. 9. The system is driven by diurnal variations in D and at steady state it follows the Lissajous curve best seen in the 06:00 figure as an oval, brown ring. At time 00:00, 06:00, 12:00 or 18:00 Q is reduced as earlier in 20 different steps. For further description see text.

The behavior is a result of several factors. The cell is sensitive to increases in D but not to decreases, so this alone creates an asymmetry.

It must be underlined that the model is very simple and that the variations in Fig. 10 most probably are too large. A closer analysis of the system kinetics combined with experiments is necessary to reveal the exact magnitude of the diurnal susceptibility variations, and the magnitude of D prior to the ischemia influences the magnitude of E and Q at the time of the ischemia. The damages due to the ischemia will change both Q and E.

So, there is an interplay between the drain variations and the inborn oscillation tendency of the system. As the system is easily agitated, continued drain variations can give rise to large transient responses that dominate the system behavior.

Discussion.

A common theme through the analysis is the behavior of the energy cogwheel. Many control systems are present in the cell and its mitochondria, but the main control is the strong and often overlooked synchronization of the different steps from nutrients to work - at least on average.

In this sense the variable E is complex. Some of the cogwheels are distributed like the proton potential, the ATP and ADP, while others like the enzymes in the Krebs cycle and the electron chain have definite localizations in the mitochondrion.

Increasing the size of a cogwheel will just make it turn slower, while the total flow is unchanged. If, on the other hand, the cogwheel in question is rate limiting, an increase, implemented by the variable Q, will also increase the total flow.

Fig. 3. Illustrates how difficult it is clinically to predict the effect of damage on a cellular level. The sharp border and the oscillations make a prediction based on clinical data complicated.

Use of statistical measures is also problematic. Even in the simple system with two variables, the complex borderline in Fig. 5 demonstrates that either a substantial analysis or a very large number of measurements is necessary to predict the borderline just approximately.

In vivo, the system is multidimensional, the borderline much more complex, and there is a certain plasticity in the nerve system (Watts et al 2018). But it should not be impossible to find a set of biomarkers that is able to predict the outcome based on an analysis like the presented.

D≠1: An increased energy drain, D, with a constant ATP production results in a decreased A=ATP/ADP ratio. This slows the maintenance of the cogwheel and makes the system more fragile. The hurdle is both the finite supply of nutrients, oxygen etc., and the fact that if just one of the cogwheels meets its maximum rotation rate, it limits the entire system.

The effect can be minimized by an acute reduction in the energy drain as in Fig. 7. On a longer time scale, the cell will increase the number of mitochondria. With the same total energy expenditure, this will increase the ATP/ADP ratio and thereby facilitate both the energy expenditure and the repair processes.

Looking at the balance between energy expenditure and blood supply, the findings demonstrate that restoring the blood supply may be insufficient, if the energy load on the cell is high. Precisely how this may influence the clinical guidelines is unclear.

D varying: The findings in Fig. 8 may seem surprising. If the normalized energy load, D, goes too fast from 1 to 3, the cell may be damaged. The background is the balance between the movement of the borderline and the movement of the point (E, Q) as a function of D and time. The change in D makes the border move from left and below the stable point (1, 1) to the border for D=3, which is left of the stable point (1.679, 1.828), but to the right of (1, 1).

The result is that both borderline and (E, Q) move towards their final state, but if the borderline overtakes the moving (E, Q), the stable point becomes out of reach, and the solution moves down to (0, 0). In this way, the result is a chase between two processes, and if the moving (E, Q) can keep the borderline behind it, the end result is stable.

The findings must be seen on the background that the energy level of a nerve cell is rather constant (Herculano-Houzel 2011), and that the level may only change 10-20% with normal activity (Lin *et al*. 2010). In this context the used change of 3 times is much.

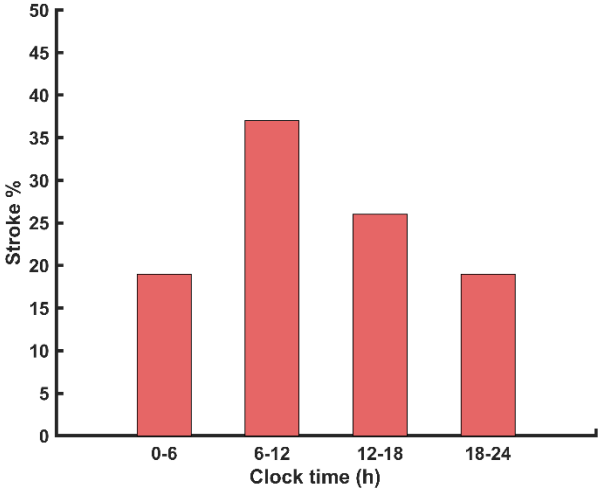

Fig. 10. Diurnal variation of stroke onset. Data from Elliot, 1998.

Diurnal variations: There is an overweight of stroke taking place in the morning. Fig. 10 shows the results of a meta-analysis on 11816 patients from 30 trials (Elliot 1998). The bars show the percentage of stroke onset in the shown time intervals.

The results accord with Fig. 9, probably because some cell damage is necessary for the diagnosis of stroke. But it must be noticed that Eliot's results include the underlying vascular events.

The change in D in the hours after the stroke is more important than the D value at the onset of the stroke. A stroke in the morning is quite severe, while one in the evening appears harmless, because the cell is passing into a silent period.

Appendix:

The appendix gives a description of the model and the normalization of the variables.

Energi production and drain: The energy-rich substances (ATP, PCr etc.) are treated as one: ATP, and the energy-low as one: ADP. With constant nutrition level, the ATP production of the cell depends on the number of mitochondrial sites (cogwheel sets) and their rotation speed. While the number of active sites may change after the hypoxia, the rotation speed depends mainly on the energy use (cogwheel 5).

The ATP use is regarded as two coupled reactions: Hydrolysis of ATP to ADP with liberation of energy, and phosphorylation of the enzymes, W, which uses this energy as:

$$ATP + W \rightarrow ADP + W\sim P,$$

where W~P is the energy-rich form of W. Taking the amount of non-phosphorylated W as a measure of the cell's energy demand, D, and assuming no saturation, the ATP use, J_D, becomes:

$$J_D = k_D \cdot ATP \cdot D.$$

where k_D is a constant.

Regarding ATP production (Cogwheel 4), the amount of active sites is called E, all rotating at the same speed. The speed depends on the balance between the concentration of ADP and ATP. To this comes that high D decreases ATP, which favors ADP. Hence, the ATP production, J_{ATP}, is:

$$J_{ATP} = k_E \cdot E \cdot ADP \cdot (1 - \varepsilon + \varepsilon \cdot D/D_{ref}),$$

where the last part corrects J_{ATP} for variations in D. The ε is a constant that depends on the actual push-pull state of the cogwheels and determines the weight of the D-correction. The D_{ref} is the energy demand at the same state that k_E is determined, typically the basic state. The use of ε ensures that the correction is one, when $D=D_{ref}$. Both ε and k_E depend to some extent also on the nutrition of the cell.

The change in ATP concentration is then:

$$\frac{dATP}{dt} = k_E \cdot E \cdot ADP \cdot \left(1 - \varepsilon + \varepsilon \cdot \frac{D}{D_{ref}}\right) - k_D \cdot D \cdot ATP$$

As the ATP/ADP system is much faster (seconds) than the breakdown and repair of the enzymes (Anson *et al* 1998, Deng *et al* 2021), it is assumed to be instantaneous. The A=ATP/ADP ratio is used as a measure of the cell's energy state is then:

$$A = \frac{ATP}{ADP} = \frac{k_E \cdot E \cdot (1 - \varepsilon + \varepsilon \cdot D/D_{ref})}{k_D \cdot D}$$

The breakdown and repair of E is described by the equation:

$$\frac{dE}{dt} = k_\alpha \cdot (M(A) \cdot Q - E),$$

where Q is the amount of enzymes etc. that repair the cogwheel, k_α is a rate constant, and M(A) is a function that describes the energy dependence of the Q-activity.

The Q describes the control system that keeps the cogwheel sites and the mitochondria functioning according to:

$$\frac{dQ}{dt} = k_\beta \cdot (M(A) \cdot F(A) - Q),$$

where F(A) is a negative feedback function, and k_α is a rate constant.

Normalization: The system is initially in a steady state, *i.e.,* dE/dt=dQ/dt=0, and to make the analysis more general, the variables are normalized by their values at the basic state, so initially they all equal one.

Indexing the reference values with a "0" gives:

$$E_0 = M(A_0) \cdot Q_0,$$

$$Q_0 = M(A_0) \cdot F(A_0),$$

$$A_0 = \frac{k_E \cdot E_0}{k_D \cdot D_0}$$

where $D=D_0$ at the basic state and $D_{ref}=D_0$, so the correction is one.

Marking the normalized values $E_*=E/E_0$ and $Q_*=Q/Q_0$ by a "*" gives:

$$\frac{dE_*}{dt} = k_\alpha \cdot \left(\frac{M(A)}{M(A_0)} \cdot Q_* - E_*\right),$$

$$\frac{dQ_*}{dt} = k_\beta \cdot \left(\frac{M(A)}{M(A_0)} \cdot \frac{F(A)}{F(A_0)} - Q_*\right).$$

The two functions, M and F are given by:

$$M(A) = \frac{M_{max} \cdot A^n}{A_{50}^n + A^n},$$

$$F(A) = \frac{M_{max} \cdot A_{50}^n}{A_{50}^n + A^n},$$

M(A) is a simple high order saturation function with a half value of A_{50}. F(A) is taken to use the same order and ensures that a high A gives a small feedback and a small A, a high feedback. M_{max} and F_{max} are the maximum values of M and F, and it is assumed that A_{50} is the same for the two functions.

As M_{max} and F_{max} cancel out, the normalized functions become:

$$M_*(A_*) = \frac{M(A)}{M(A_0)} = \frac{A^n}{A_{50}^n + A^n} \cdot \frac{A_{50}^n + A_0^n}{A_0^n} = \frac{A_*^n \cdot (1 + K_*^n)}{K_*^n + A_*^n},$$

$$F_*(A_*) = \frac{F(A)}{F(A_0)} = \frac{A_{50}^n + A_0^n}{A_{50}^n + A^n} = \frac{(1 + K_*^n)}{K_*^n + A_*^n},$$

where $A_* = A/A_0$ and $K_* = A_{50}/A_0$. Unless specified otherwise, the parameters are $K_* = 0.7$, $n = 7$. The rate constants are taken to be $k_\alpha = 0.5\ h^{-1}$ and $k_\beta = 0.25\ h^{-1}$. The constants represent a large spectrum of time scales and could be smaller, but with the simple system of two differential equations, it just corresponds to a scaling of time.

The variable D_{ref} is taken to equal D_0 at the basic state, and D is assumed not to be influenced by the small energy demand for the production of E and Q, but it may vary with time due to other metabolic events in the cell.

In the result and discussion chapters the parameters are all normalized, so the "*" are removed.

Hill coefficient: The used Hill coefficient of 7 may seem rather large, but in the first place, the rebuilding of E includes many ATP-dependent processes, each of which may have a high Hill coefficient. The concerted action will include a high degree of cooperativity.

In the second place, the use of a Hill coefficient is always an approximation. Take for example a simple binding of n molecules of ligand, L, to a receptor, R:

$$n \cdot L + R \leftrightarrow L_n R$$

This gives typically a receptor occupancy, O, of

$$O = \frac{L^n}{K_D^n + L^n},$$

where K_D is a constant and n is the Hill coefficient. But this requires that all n ligand molecules hit at the same time, which is not probable or at least extremely rare. Instead, the different options, L_1R, L_2R ...$L_{n-1}R$, will exist for a period, and only if a total of n molecules hit during this period, the L_nR can be created (Prinz 2010). Generally, the occupancy is therefore:

$$O = \frac{L^n}{P(L) + L^n},$$

where P(L) is a polynomial in L up to degree n.

The use of n=7 is thus a compromise between a high degree of cooperativity in the many actions of ATP and the moderating effect of the polynomial.

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