Course: Computational Systems Biology BT5240 Studying the effects of Molecular Interactions and Ageing on the Glymphatic System using Dyanmic Modelling

Final Report
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Abstract

The brain has enormous energy requirements, and consequently, it produces much of the metabolic waste, including beta amyloids. The glymphatic system is the waste clearance system of the brain, which is predominantly active during sleep. Cerebro-spinal fluid (CSF) flushes/transfers the metabolic waste from brain tissues to blood vessels via diffusion through the blood-brain barrier (BBB). Substances like Caffeine and Rifampicin have been shown to provide increased clearance of metabolic waste. In this project, we propose an ODE-based dynamic model describing the working of Glymphatic system that accounts for the concentrations and accumulation of metabolic waste during the sleep-wake cycles and the cleansing mechanism of metabolic waste by Cerebro-Spinal Fluid (CSF). Using this dynamic modelling approach, we aim to find out the effects of ageing on the clearance of β Amyloids. These changes lead to increased accumulation of β Amyloids. We also aim to find out the long term effects of the irregular sleep-wake cycles and sleep derived conditions that might lead to beta-amyloid accumulation, which can have potential neurodegenerative implications. Building on this, Amyloid Precursor Protein (APP) is the precursor protein to Amyloid β_{40} and Amyloid β_{42}. In cases of Down Syndrome and Aluminium exposure, our model suggests early-onset Alzheimer's Disease due to higher accumulation of Aβ. Additionally, we have modelled the action of Caffeine and Rifampicin on Glymphatic system and understand the underlying mechanisms. Our results suggest a lower accumulation of Aβ in the long run, with the intake of Rifampicin and Caffeine. Similar modelling-based studies can provide a quantitative basis for identifying drug targets and preliminary drug screening.

Introduction

The human brain has very high energy per mass requirement. Therefore, it contributes to produces large amounts of waste metabolites such as Amyloids β protein. Amyloids β and other waste metabolites can

be neurotoxic. Amyloid β toxicity has also been linked to various neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease (1). Therefore, The clearance of these interstitial solutes is crucial for tissue homeostasis. The clearance of these waste metabolite is done largely by a recently discovered mechanism called the Glymphatic system.

The Glymphatic System, a macroscopic waste clearance system that efficiently utilizes the perivascular tunnels of the astroglial cells to clear up solute proteins and waste metabolites. It also helps in brain-wide distribution of glucose, amino acids, growth factors, neuromodulators and lipids. However, glymphatic system is predominantly active during sleep (2). The disruption of the Glymphatic system has shown to be linked to Chronic traumatic encephalopathy (3), Diabetes-Induced Dementia (4) and even several neurodegenerative diseases such as Alzheimer's Disease (5). Understanding the working principles of Glymphatic system can potentially help us to understand the transport mechanisms within the brain.

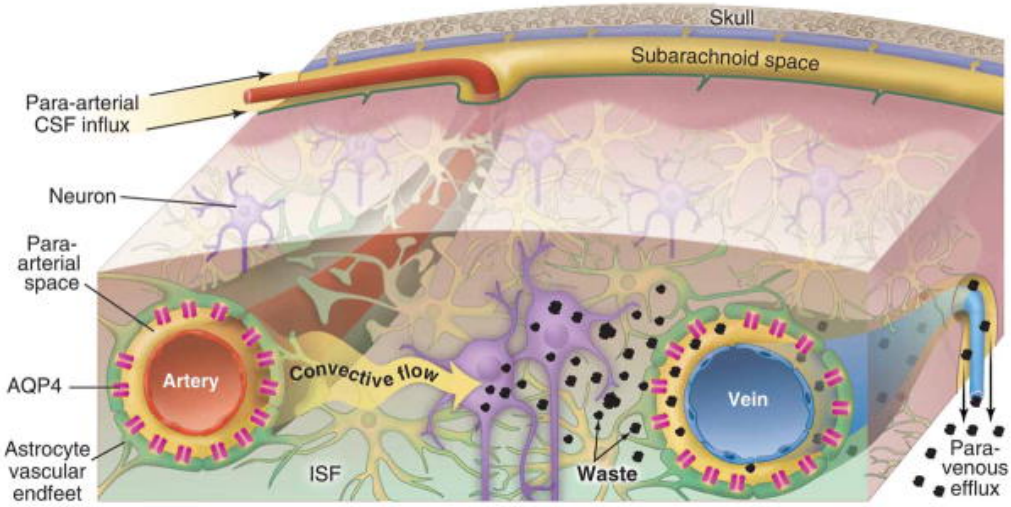


Figure 1: **Overview of the circulation of CSF and ISF through the glymphatic pathway**
The bulk flow of CSF into brain specifically within the perivascular spaces of penetrating arteries drives interstitial metabolic waste products toward perivenous spaces, and ultimately from the cranium via several post-glymphatic clearance sites, including arachnoid granulations, meningeal lymphatic vessels, and along with cranial and spinal nerve roots. Reproduced from Plog et al. (6)

There are several mathematical models for modelling various aspects of the glymphatic system (6), (7), (8). In our work, we tried to account for the sleep cycles on the shorter time scale of hours and its implication over the years. We have modified the model proposed by Kyrtsos et al. (8) to include the short term effects of the sleep-wake cycle. We have accounted for irregularity in sleep and compared it with a model with regular sleep.

It is known that Aβ is a product of Amyloid Precursor Protein (APP) which undergoes degradation. APP production is altered in cases such as exposure to Aluminium and case of Down Syndrome (9). C99 is produced by the action of β secretase on APP. C99 is further cleaved by γ secretase to form either Aβ_{40} or Aβ_{42} (10).

Lastly, we examined the role of drug-like molecules that have shown to enhance the clearance rate of Amyloid β. Caffeine and Rifampicin have been shown to enhance the Amyloid β clearance (11). This is mediated by P Glycoprotein (PGP), and Lipoprotein Receptors 1 (LRP1) mediated process. We have accounted for the effects of Caffeine and Rifampicin on LRP1 and PGP. This way, we have been able to study the effects of Caffeine and Rifampicin in our work.

Methods

We have adopted the three-compartment model equations from Kyrtsos et al. (8). It is assumed that the model is made up of three compartments, namely, Brain parenchyma, where the neurons, microglia cells are present. Neurons produce β Amyloids and which is taken up by microglia cells. β Amyloids gets transported into perivascular space which is modelled by the mass transfer equation. Later on, it is taken up by the blood vessels near brain parenchyma.

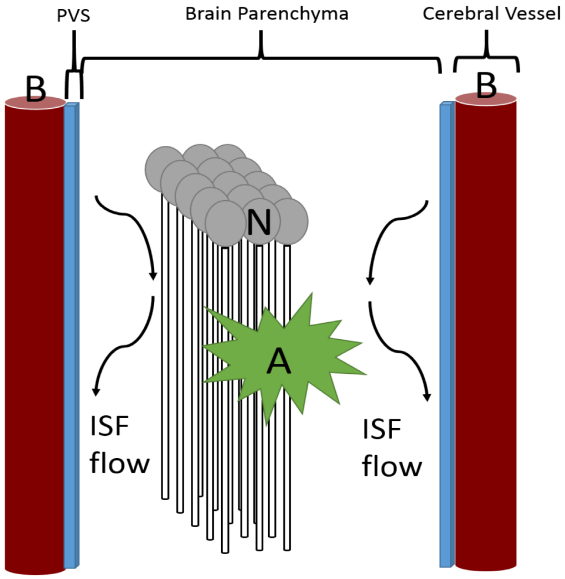


Figure 2: Three compartment model of Glymphatic System adopted from Kyrtsos et al. (8).

Sleep-Wake cycle modification in Three Compartment Model

The parameters are adopted from the Kyrtsos et al. (8) study. The parameters for sleep and wake conditions are determined, such as the average value of the parameter remains constant (as given in the study). The fold change is taken into account (k_{factor}).

$$k_{factor} = \frac{k_{awake}}{k_{sleep}}$$

$$k_{awake} = \frac{24 * k * k_{factor}}{24 + (\text{awake hours}) \cdot (k_{factor} - 1)}$$

$$k_{sleep} = \frac{24 * k}{24 + (\text{awake hours}) \cdot (k_{factor} - 1)}$$

$$v_{factor} = \frac{v_{awake}}{v_{sleep}}$$

$$v_{awake} = \frac{24 * v * v_{factor}}{24 + (\text{awake hours}) * (v_{factor} - 1)}$$

$$v_{sleep} = \frac{24 * v}{24 + (\text{awake hours}) \cdot (v_{factor} - 1)}$$

Dynamics of β Amyloid-40,42 in Brain Parenchyma

$$\dot{C_1} = [\begin{cases} -v_{sleep}\frac{dC_1}{dx} + k_{3sleep}k_4N(t) - k_5M(t) - k_{7_a}C_1(t) & \text{Sleep Condition} \\ -v_{awake}\frac{dC_1}{dx} + k_{3awake}k_4N(t) - k_5M(t) - k_{7_a}C_1(t) & \text{Wake Condition} \end{cases}] \tag{1}$$

Dynamics of β Amyloid-42 in Perivascular Space

$$\dot{C_4} = [\begin{cases} v_{sleep}\frac{dC_1}{dx} - k_6(\frac{LRP(t)}{LRP_0})C_2(t) - k_7C_2(t) & \text{Sleep Condition} \\ v_{awake}\frac{dC_1}{dx} - k_6(\frac{LRP(t)}{LRP_0})C_2(t) - k_7C_2(t) & \text{Wake Condition} \end{cases}] \tag{2}$$

Sleep Irregularities and Deprivation

Case (i): Sleep Deprivation

This model is used to simulate the glymphatic activity for a regular as well as irregular patterns of sleep. For the case of regular sleep, the "awake hours" in the above equations is a constant value of 16 hours (8 hours of sleep daily). To account for sleep deprivation, the simulation time for the time of sleep is changed to randomly generated integers between 0 and 8..The distribution is uniform. Therefore, the missed hours of sleep are not compensated for, leading to a sleep-deprived condition. However, the sleep and awake values of velocity and rate constants are still the same as that for regular sleep to maintain biological consistency.

Case (ii): Irregular Sleep

The model used for simulating Aβ accumulation for regular sleep cycle is awake for 18 hours (6 hours of sleep daily). The time of sleep is a random integer generated from a uniform distribution between 2 and 10 ($\mu = 6$). This distribution makes sure that the mean number of hours of sleep is the same as that of regular sleep. In other words, the lost sleep on one day is compensated for on another day leading to an irregular sleeping cycle. The sleep and awake values of velocity and rate constants are still the same as that for regular sleep to maintain biological consistency.

APP production and related studies

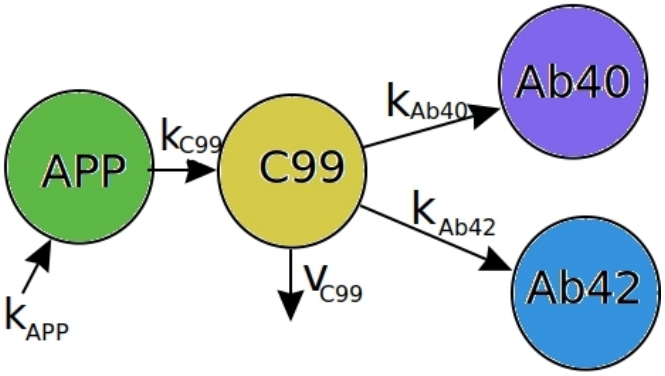


Figure 3: Additional two step processes for modelling Amyloid β production from APP.

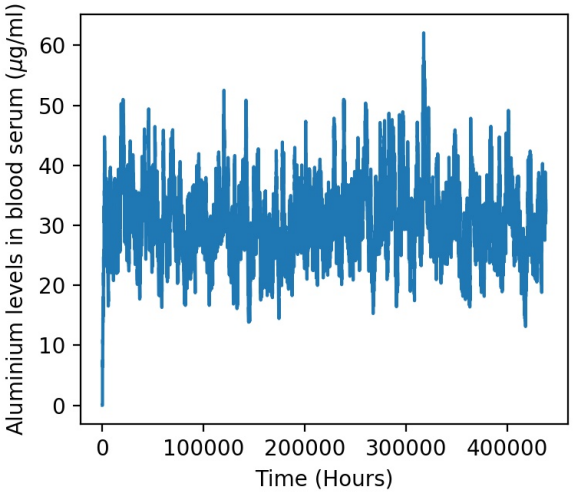


Figure 4: Variation of Aluminium in blood serum.

Accounting for APP production in our model is essential to study the effects Down Syndrome and Aluminium exposure. A two step formation of Amyloid β is taken into account as shown in the figure approach is undertaken as shown in figure 3. Parameters for the additional two steps are obtained from (12).

APP gene occurs on chromosome 21. We used BLAST to search for other gene duplications within chromosome 21 or in other chromosomes, but these duplications are rare. However, in the case of Down Syndrome, there is an abnormal presence of 3 copies of Chromosome 21 (9). This increases the gene dosage of APP above the basal levels. In order to model this effect, we increased the production rate of APP gene to 1.5 times to its previous value.

Aluminium is said to be a potential trigger for enhanced Aβ production. It has been shown that Inflammation, presence of Aluminium ions, a head injury can potentially trigger the immune response and activate HIF1 and NF-κB (13), (14). HIF1 an NF-κB further bind to the promoter region of APP and increase its production. This process is modelled by a periodic stochastic intake of Aluminium. The intake leads to increase of 2.5 μg/ml in blood serum (This value is obtained by getting a consistent long-range average value for Al^{3+} ions in blood serum from Krewski et al (15)). The half-life of Aluminium is 4-6 weeks in human body (16). The variation is shown in figure 4. Aluminium above a particular threshold (20μg/ml) would saturate, and APP production would increase to 1.2 times the normal value. For the same value between 20μg/ml and 10μ, g/ml would lead to a proportional increase in APP production while no particular effect for values less than 10μg/ml.

Effects of Rifampicin and Caffeine

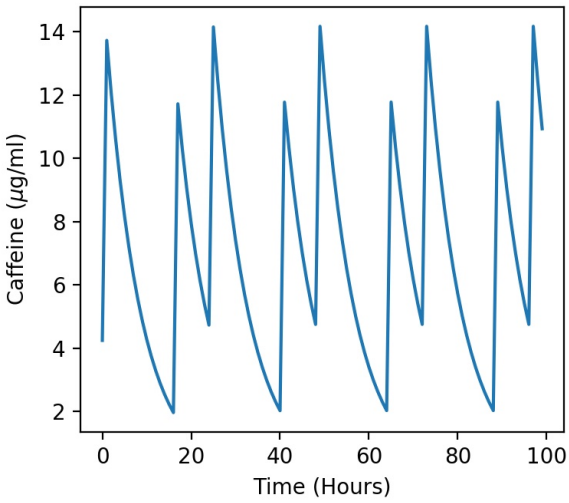


Figure 5: Caffeine concentration dynamics.

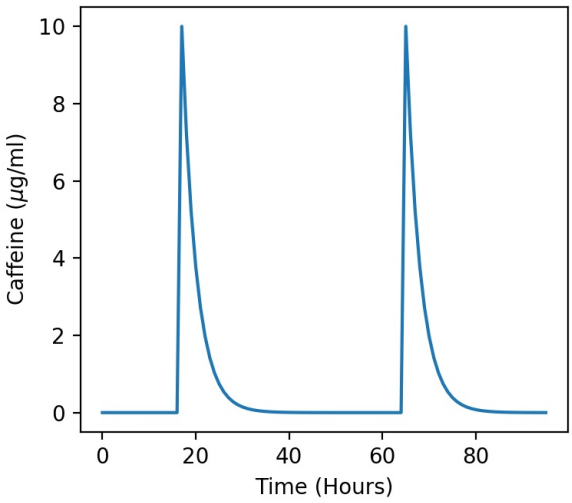


Figure 6: Rifampicin concentration dynamics.

Caffeine elevates the P-glycoprotein (PGP) levels in the blood-brain barrier (11). PGP acts as a transport protein and enhances the Amyloid β clearance rate by enhancing the flow rate of CSF(17). This effect is modelled by enhancing the flow rate in our compartmental model. There are some evidences that Caffeine also elevates the blood pressure (18), which enhances the blood flow rate. Caffeine has a half-life of 5 to 6 hours (19) in the human body. Caffeine intake is twice a day with a probability of 0.9. A cup of coffee (95 mg of Caffeine) leads to an increase of Caffeine in the brain 10 μg/ml in the brain by our estimate. Fig.5 shows the input caffeine dynamics for the compartment model, where each peak represents a pulse of caffeine intake.

Rifampicin elevates P-glycoprotein (PGP) levels as well as LRP1 levels as shown in the study conducted by Qosa et al. (11). The amount to which the PGP and LRP1 are elevated is obtained from Qosa et al. LRP1 levels are explicitly modelled; however, LRP1 has unusually low degradation rate and a half-life of 24 hours (20). Since Rifampicin can have side effects and adverse effects on gut microbiota (21), the amount of Rifampicin uptake has been varied with the highest frequency being once a day. The half-life of Rifampicin is about 2.5 hours (22). Fig.6 shows the input Rifampicin dynamics for the compartment model, where each peak represents a pulse of Rifampicin intake. Both Caffeine and Rifampicin concentrations follow first-order decay kinetics in the blood as seen in the figures 5,6.(19; 22)

Results

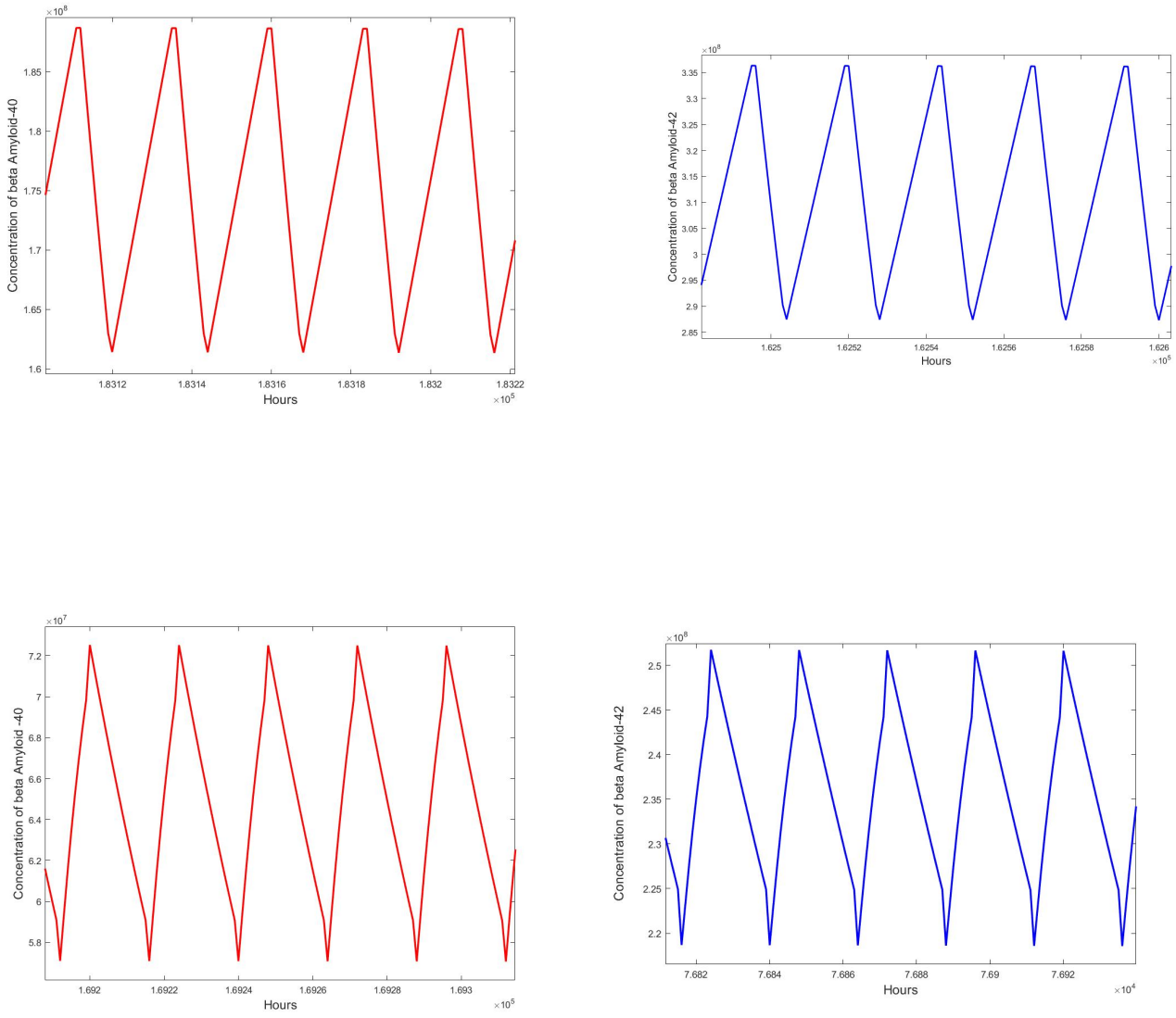


Figure 7: Variation in β Amyloid - 40 (left top) and 42 (right top) concentration in the brain parenchyma during sleep wake cycle.
Variation in β Amyloid - 40 (left bottom) and 42 (right bottom) concentration in the perivascular space (Intersitial Fluid and Cerebrospinal Fluid) during sleep wake cycle.

The objective this model was to study the variation of the amount of metabolic waste, notably, β Amy-

loids, present in the brain by studying the glymphatic pathways. The model is also aimed at studying the effects of ageing on the accumulation of β Amyloids by modelling the perivascular pathway and transport through the Blood-Brain Barrier by LRP-1. Ageing also causes a steady decrease in the number of cells (neurons, microglia, and endothelial cells). These effects have also been incorporated in the model.

Fig.7 shows the variation of β Amyloids in the brain parenchyma and the perivascular space, with respect to time in the scale of days. In this study, we can observe the effects sleep has in the functioning of the glymphatic system. During the night time, the day time, the flow of the ISF and CSF are slow and hence lead to accumulation of the solutes. During the time of sleep, the flow rates significantly increase, and all the accumulated waste is cleared given enough time. These trends are in accordance with the experimental study by Erin L. Boespflug and Jeffrey J. Iliff (23). Perturbations on this part of the model will provide insights into both the short term and long term effects of insufficient sleep on the waste accumulation and function of glymphatic system.

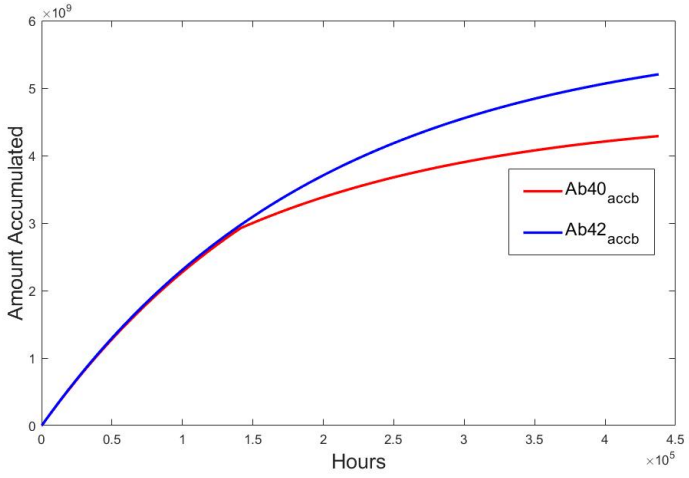


Figure 8: Accumulation of β Amyloids in brain parenchyma over a period of 50 years.

Fig.8. Demonstrates the accumulation of the β Amyloids over a period of fifty years, taking the sleep-wake cycles into account. As expected, we observe that the accumulation of waste increases in brain parenchyma. Fig.9. Depicts the gradual decrease in the number of functioning cells and LRP-1 with ageing. The LRP-1 receptors play a crucial role in transport through Blood-Brain Barrier (8). Hence, it can be inferred that the transport through the Blood-Brain Barrier is also affected by ageing.

Sleep Irregularities and Deprivation

We analysed the dynamics of the β Amyloid accumulation under irregular sleeping patterns and sleep-deprived conditions. We observed the accumulation of these metabolites both in the brain parenchyma and the perivascular region. Fig.10 shows the comparison between the β Amyloid accumulation in the regular sleep cycle and a sleep-deprived state over a period of 50 years. It is clearly evident from the

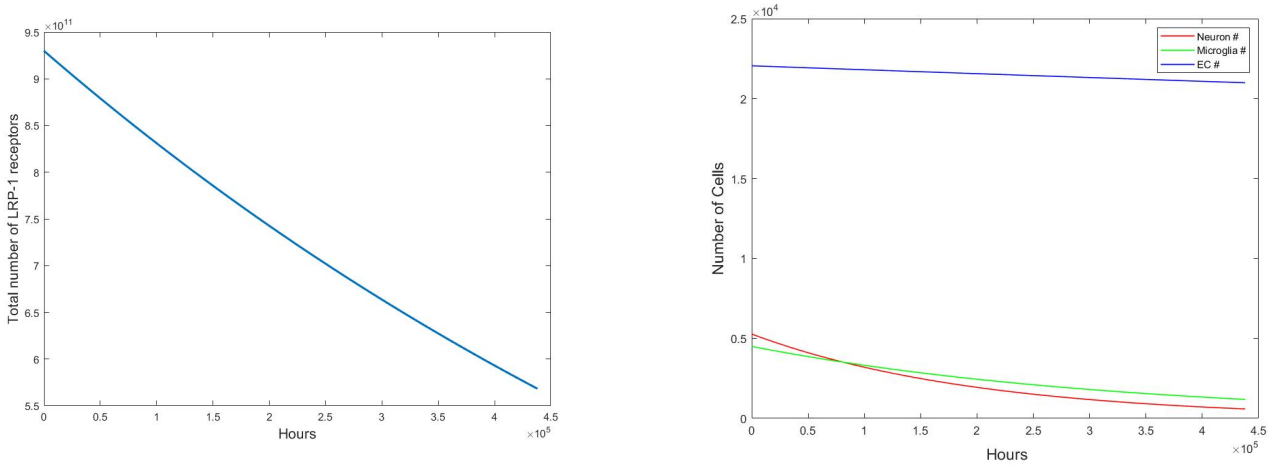


Figure 9: The decay of LRP-1 receptors with aging(left). The variation of numbers of different cells involved in the functioning of glymphatic system (right).

analysis that irregular sleeping habits lead to significantly higher accumulation of β Amyloids. In the case of irregular sleep, we obtained similar results as the deprivation case. It is important to note that even though the mean of the number of hours of sleep is the same, the heterogeneity in the number of hours of sleep leads to increased production of $A\beta$ in the long run. There is a higher accumulation of $A\beta$s in brain parenchyma compared to Perivascular space which is similar to the results found in the case of deprivation.

(24; 25). This shows that irregular sleeping habits make us prone to higher chances of neurodegeneration, leading to Alzheimer's Disease.

Note that both the results qualitatively agree with the clinical study conducted by Zee et al (26) and Lim et al (27). The irregularity in sleeping patterns can potentially lead to Aβ accumulation which paves the way for Alzheimer's Disease. However, these papers also mention about the irregularity seen after the onset of AD which our model does not account for.

APP production, Down Syndrome and Exposure to Al^{3+} ions

The results shown in figure 12, demonstrate the effect of Down Syndrome and HIF & NF-κB mediated effects of Al^{3+} ions on increased production of APP. Although the waste clearance is dependent on the amount of waste present, the amount of deposited Amyloid β is clearly highest in Down Syndrome as seen in figure 13. This can potentially lead to accumulation of Amyloid β and lead to the onset of neurodegenerative diseases. This result is consistent with the experimental results by Head et al., which showed

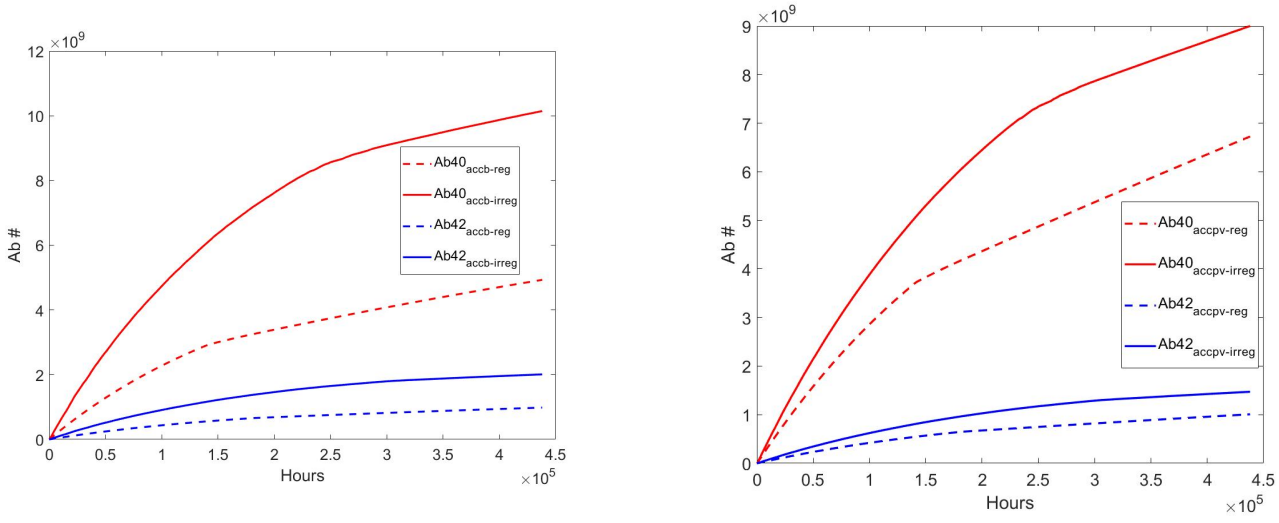


Figure 10: The accumulation of β Amyloids 40 and 42 in the brain parenchyma(left). The accumulation of β Amyloids 40 and 42 in the perivascular region (right). Note that the accumulation for sleep irregularity is higher in the brain parenchyma. Using Case (i) Sleep Deprivation

early onset of Alzheimer-like behaviour in children suffering from Down Syndrome and attributed it to the accumulation of Amyloid β (28).

As seen in figure 13, Aluminium exposure even in moderate amounts, leads to a higher accumulation of Amyloid β and elevates the risk of Dementia and Alzheimer's. Our model quantitatively confirms the established observation that Aluminium promotes Amyloid β aggregation at an earlier age and can lead to Dementia and eventually, Alzheimer's (29), (30), (31).

Effects of Rifampicin and Caffeine

Caffeine

With the input caffeine as described in the Methods section, the simulations were performed to study the accumulation of Amyloid β under the influence of Caffeine. Fig.14 shows the comparison of these accumulations with and without Caffeine intake.
From the plots comparing the accumulation of Amyloid β, we can see that under the effect of Caffeine, the accumulation in the brain parenchyma goes below the values of accumulation without Caffeine because of the increased flow of CSF. But this does not continue in the perivascular region. From the work of Qosa et al., it is known that Caffeine does not affect the LRP-1 receptor number (11). It is known that LRP-1 receptors play an important role in the clearance of metabolites through the Blood-Brain Barrier (32).

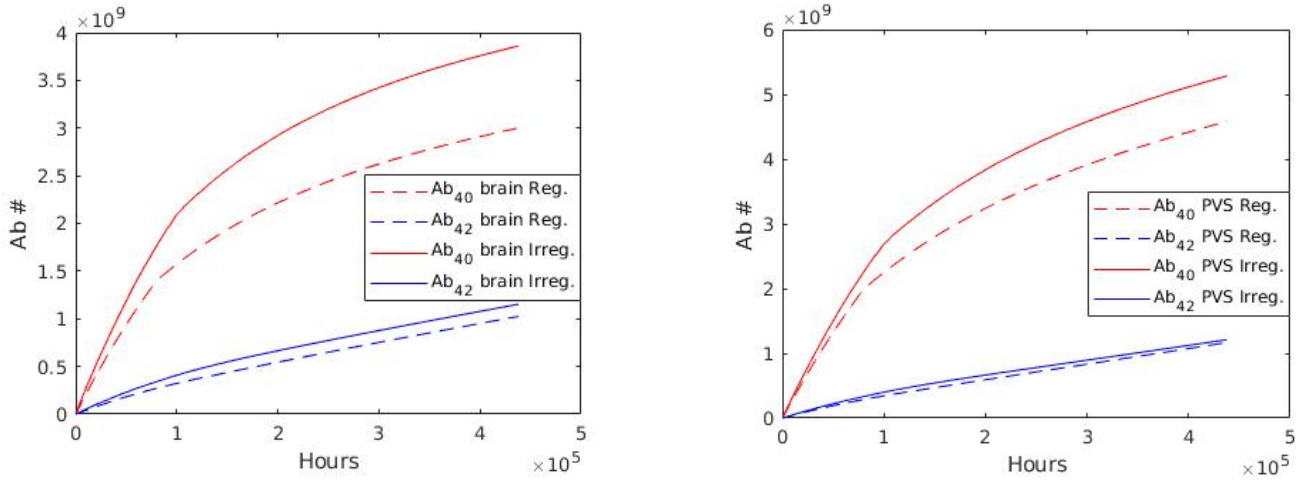


Figure 11: The accumulation of β Amyloids 40 and 42 in the brain parenchyma(left). The accumulation of β Amyloids 40 and 42 in the perivascular region (right). Note that the accumulation due to sleep irregularity is higher in the brain parenchyma. Using Case (ii) Sleep Irregularity

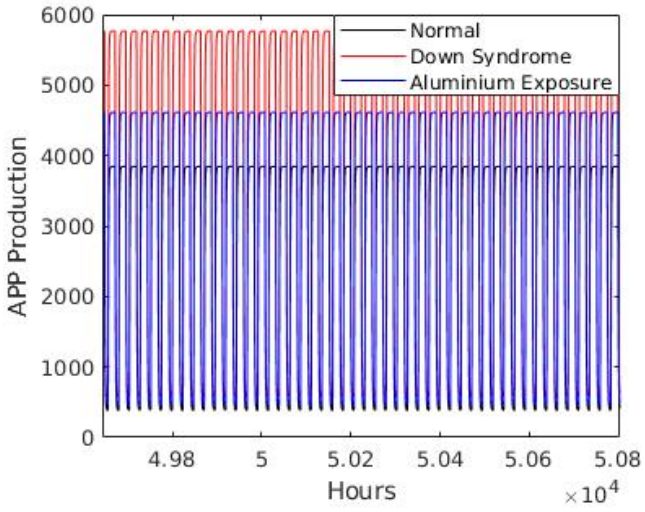
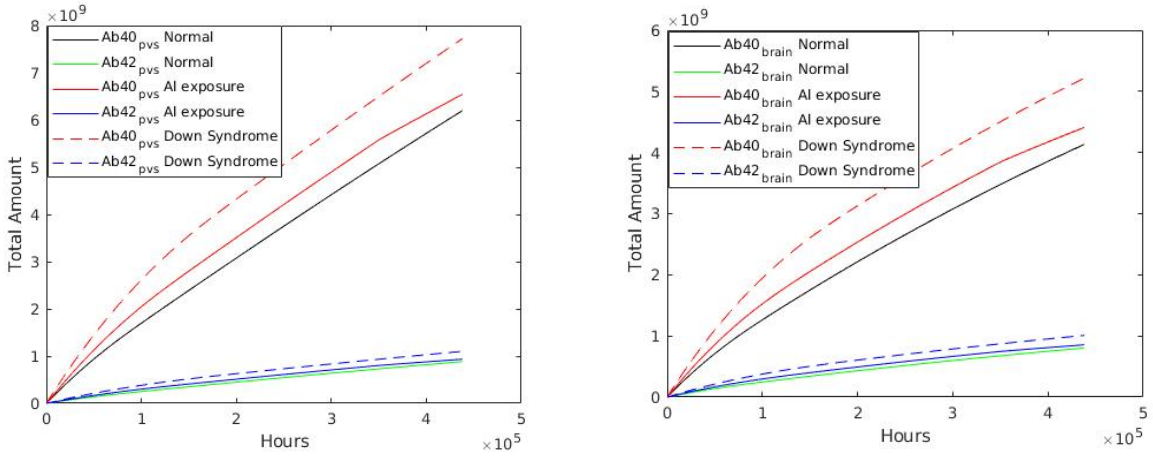


Figure 12: APP variation under different conditions in the order of days

Hence all the additionally cleared metabolites in the brain accumulate at the perivascular region before crossing the BBB and entering the bloodstream. This points to the need for a better boosting mechanism to clear the metabolites.



(a) Perivascular Space

(b) Brain Parenchyma

Figure 13: Deposits of Ab40 and Ab42 in (a) Brain Parenchyma and (b) Perivascular Space, in Normal, Down Syndrome and Aluminium Exposed Condition

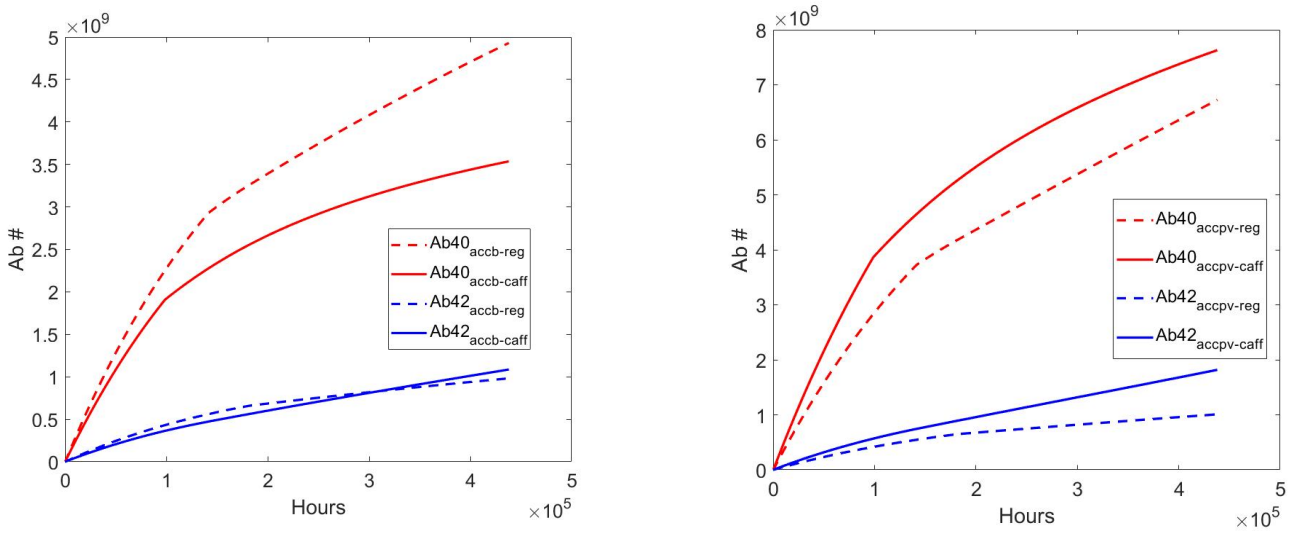


Figure 14: The accumulation of Amyloids β 40 and 42 in the brain parenchyma(left). The accumulation of Amyloids β 40 and 42 in the perivascular region (right). In both cases, the dotted line shows the accumulation in the absence of Caffeine and the solid line in the presence of Caffeine

Rifampicin

The model has been simulated for a variable frequency up to a maximum of once a day. The work done by Qosa et al. (11) highlights that the change in the number of LRP-1 receptors can increases by two-folds due to the presence of Rifampicin in mouse brain models. This fact has been accounted for in our work. Fig.15 shows both the short term and long term effects of Rifampicin intake on the LRP-1 receptors.

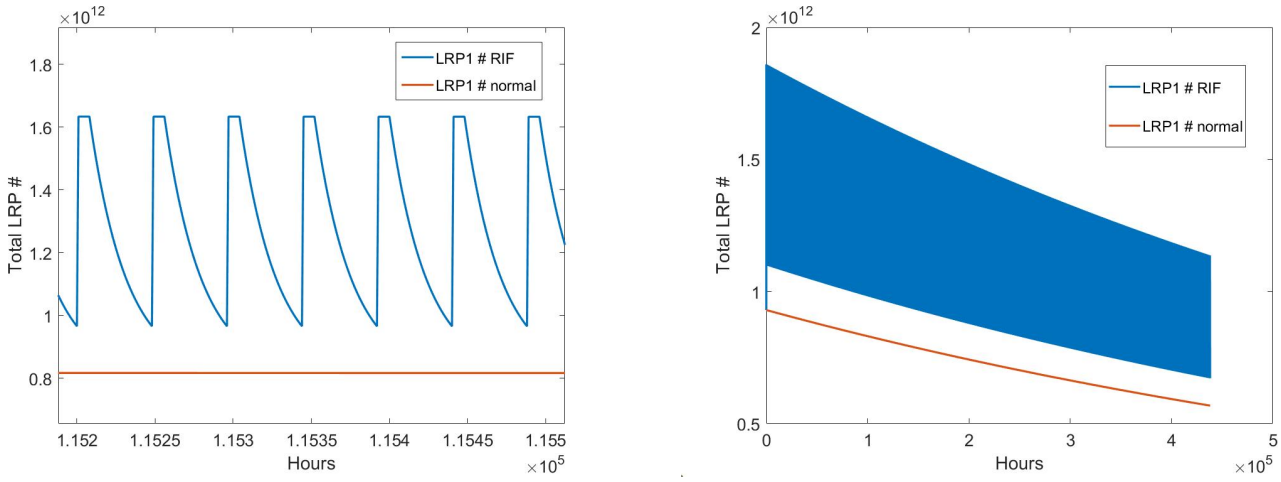


Figure 15: The number of LRP-1 receptors with and without Rifampicin intake on a daily basis(left). The number of LRP-1 receptors with and without Rifampicin intake over a period of 50 years (right).

After modelling the effect of Rifampicin, we studied the accumulation of Amyloid β in the presence and absence of Rifampicin. As shown in fig.16, the accumulation of Amyloid β goes down in the long term. This is because of two factors governing this change. Firstly, it is a rise in P-Glycoprotein levels which facilitates a higher flow of CSF through the Blood-Brain Barrier and Blood CSF Barrier. Secondly, it is the rise in LRP-1 receptors that facilitates better clearance of A$\beta_4$0. Moreover, it can be seen that the decrease in accumulation is higher in perivascular space which is a reflection of the fact that the change in LRP-1 receptor number is a major effect of Rifampicin.

Discussions

Model Assumptions

The study of Glymphatic system involves the clearance of all metabolic waste from the brain. However, we have considered only β Amyloids in this model due to the availability of data. Furthermore, this

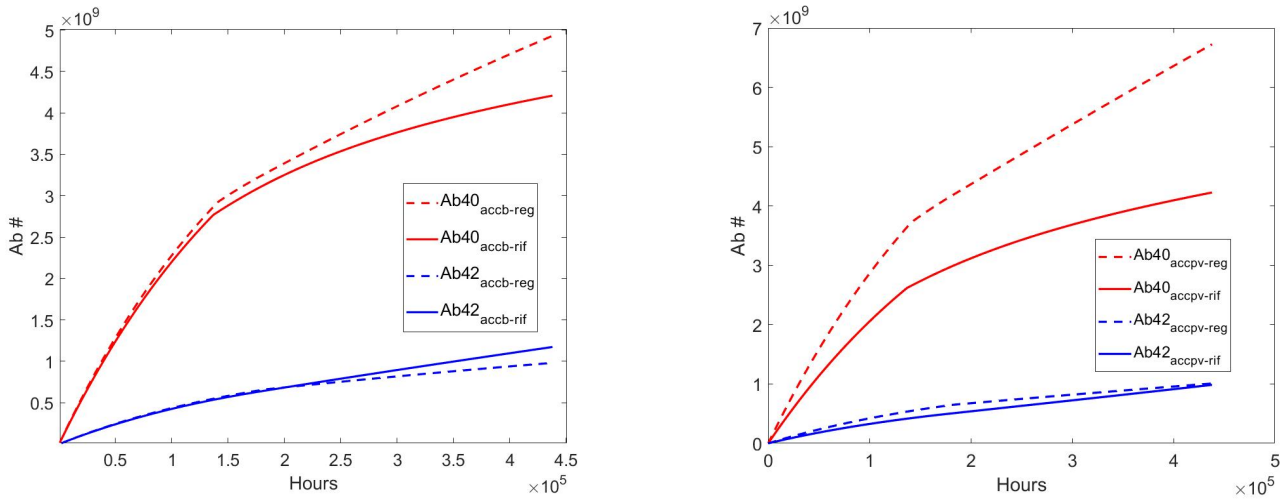


Figure 16: The accumulation of Amyloids β 40 and 42 in the brain parenchyma(left). The accumulation of Amyloids β 40 and 42 in the perivascular region (right). In both cases, the dotted line shows the accumulation in the absence of Rifampicin and the solid line in the presence of Rifampicin

model has been adopted from the work of Kyrtsos et al. (8) and hence all the biological assumptions that have gone into developing the three-compartment model follow. This includes the estimated parameters for this model. Besides this, The simulations have been performed assuming the persons sleep for eight hours every day. In our analysis related to Alzheimer's disease, we have taken the ef in effects solely of β Amyloids based on studies by Rajasekhar et al. (1).

Sleep Deprivation and Irregular Sleep

We have shown the working of glymphatic system during regular, irregular and deprived sleeping patterns. It seldom happens in reality that we have a strictly regular sleep cycle. But we try to compensate for this by adjusting our sleep at later point of time. However, our simulations show that this does not compensate for the function of the glymphatic system, thereby causing higher accumulations of $A\beta$. Hence, the effects of irregular sleep cycles and sleep deprivation both in the long term and short term in terms of the β Amyloid accumulation rates in the brain are brought to notice through our model providing insights into healthy sleeping habits.

Amyloid Precursor Protein and related studies

Amyloid β production is governed by the production of APP (Amyloid Precursor Protein). It is evident from our results that increased production of APP can lead to early onset of neurodegenerative diseases.

This is characterised in the case of Down Syndrome and Aluminium exposure. A study can be done to identify other potential triggers of APP production. APP can be a drug target for the treatment of Alzheimer's disease. Additionally, in the case of Down Syndrome patients, APP targeting drugs can be used to keep the neuronal degeneration and Amyloid β aggregation.

Our model can be used to identify and quantify the effects of drug targets and can be useful in preliminary screening.

Caffeine or Rifampicin?

We have successfully demonstrated that the model can be used to predict long term effects of the intake of Caffeine and Rifampicin on the Glymphatic System(Caffeine:(33; 34; 35; 36); Rifampicin:(37; 38)). Kryt-sos et al. have accounted for the LRP-1 level in their model (8). LRP-1 level in Endothelial cells helps in the clearance of Amyloid β through the Blood-Brain Barrier. Caffeine is shown to affect the flow of CSF, increasing the metabolite waste clearance in the brain parenchyma tissues. But, Caffeine does not affect the LRP-1 receptors (11). From the analysis performed, we have infered that the Caffeine intake results in a bottleneck situation near the perivascular region, however, it can still be beneficial as depo-sition in perivascular region is not as harmful as it is in brain parenchymal tissues. Moreover, we can observe that the accumulation in the perivascular region is closer to the normal value than that in the brain parenchyma. Hence, we infer that Caffeine is effectively helping by reducing the accumulation in the brain tissue. Moreover, the side effects of the dosage used in the model are minimal (39).

On the other hand, Rifampicin shows great results in reducing the accumulations in both brain parenchyma as well as perivascular space. This is possible because it not only increases the flow rate of CSF but also approximately doubles the number of LRP-1 receptors making it an efficient mechanism to increase Amy-loid β clearance (11). However, it has been shown that the side effects of Rifampicin are more serious than those of Caffeine used at a low dosage (21). Hence, the choice between a better mechanism to clear the accumulation of β Amyloids comes down to this trade-off between the speed of clearance and the side effects. This can be the starting point for studies considering these substances as a potential treatment for different stages of Alzheimer's disease.

Conclusion

We have successfully demonstrated our model for the working of Glymphatic system across different timescales from days to years. We have been able to show the effects of sleep irregularities, sleep depriva-tions, Aluminium exposure and the case of Down Syndrome, all of which are triggers of Aβ accumulation and pave the way for early onset of Alzheimer's Disease. As a measure against this neuro-degeneration, we have explored the potential therapeutic substances and demonstrated the working of Caffeine and Rifampicin, which are shown to enhance the clearance of Aβs.

Supplementary Material

Code Availability

The MATLAB code used to simulate this model is available through the following link.
`https://github.com/burhan1118/Modelling-Glymphatic-System`

References

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