Physiological Aspects of Astrocytes in Alzheimer's Disease

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***Abstract*—Numerous cell types interact intricately with Alzheimer's disease (AD), and astrocytes have emerged as key participants in AD progression as well as possible treatment approaches [1,2]. With an emphasis on their dual functions in neuroprotection and neurotoxicity, this study investigates the physiological features of astrocytes in AD [1-4]. We study transcriptional changes throughout disease progression, the intricate relationships between neurons, astrocytes, and microglia, and astrocyte dynamics around amyloid-β plaques using sophisticated imaging techniques, single-cell RNA sequencing, and mathematical modeling. According to our research, two separate astrocyte subtypes—A1 harmful and A2 helpful—each plays a unique functional role in AD pathogenesis. We show that the development of the disease is greatly impacted by calcium dysregulation in astrocytes and suggest that the Piezo1 channel may be a viable target for treatment. The crucial involvement of astrocyte-mediated processes is highlighted by mathematical modeling of amyloid-β dynamics, which offers quantitative insights into the production and clearance mechanisms.**

Keywords: *Alzheimer's Disease (AD), Astrocytes, Neurodegenerative Diseases, Reactive Gliosis, Amyloid-β*

I. INTRODUCTION

A key sign of neurodegenerative diseases is reactive gliosis, which involves functional and structural changes in astrocytes and microglia [1-4]. Astrocytes contribute to brain functions in the central nervous system, and the pathogenesis of neurodegenerative diseases like Alzheimer's disease (AD) [5]. Considering the current disease stage, astrocytes play a binary role in AD including neuroprotection and neurotoxicity [6]. Reactive astrocytes in AD brains are detected around the Amyloid-β (Aβ) plaque formation/clearance, neuroinflammation, and synaptic dysfunction and are present throughout AD progression [7-11]. Recent progress in transcriptomics has improved the modern understanding of astrocytes and their molecular pathways in AD [12]. The advanced mathematical modeling of interactions between neurons, astrocytes, microglia, and amyloid-β dynamics further enhances the current knowledge in this field [13].

Amyloid-beta (Aβ) oligomers and plaques both play critical roles in the progression of Alzheimer's disease. Aβ oligomers, the smaller, soluble aggregates of Aβ, are highly toxic and disrupt neuronal function early in the disease. They interfere with synaptic transmission by binding to synaptic receptors, such as NMDA and AMPA receptors, impairing long-term potentiation (LTP) and promoting synaptic loss, which leads to memory deficits [35-36]. Oligomers also induce oxidative stress, calcium dysregulation, and mitochondrial dysfunction, triggering neuronal apoptosis [37]. Aβ plaques, the larger, insoluble extracellular deposits, contribute to chronic neuroinflammation by activating microglia and astrocytes. This persistent activation promotes the release of pro-inflammatory cytokines and reactive oxygen species (ROS) [38].

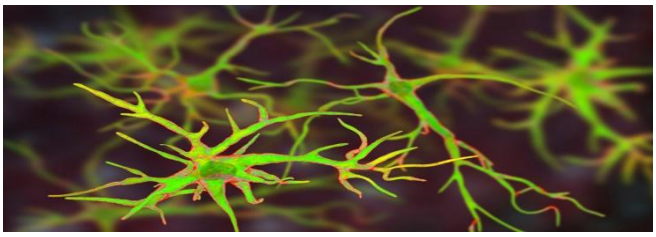


Figure 1, 3D Detailed Image of Astrocytes [39]

II. LITERATURE REVIEW

Earlier studies show astrocytes as passive supportive glial cells [14], however, there is a remarkable gap in our understanding of their different roles in the way they affect the neural circuits they are part of. Progress in this field, especially in understanding astrocytes' role in neural disorders and their interaction with neuronal circuits, has been constrained by the absence of quantitative models. This is attributed to the lack of specialized tools required to understand astrocytes and describe their dynamic relationship with disease progression [15-19].

Neurotoxic astrocytes are important in neurological disease development, and the exceeding microglial activity inhibits their production which might break the progression of AD. However, no direct therapies for these astrocytes have been developed yet, due to the limited understanding of the molecular mechanisms governing astrocyte-microglia interaction in different stages of AD [20-22]. Growing evidence links *tau* pathology and neurofibrillary tangles in Alzheimer's disease to metabolic dysfunction in astrocytes [23-25]. However, this dysfunction may have neuroprotective effects and might not be harmful. Whether it is beneficial or detrimental in humans remains uncertain, leaving the role of astrocytes in Alzheimer's disease unclear [26-29].

III. METHODOLOGY

A. *Multiphoton Microscopy*

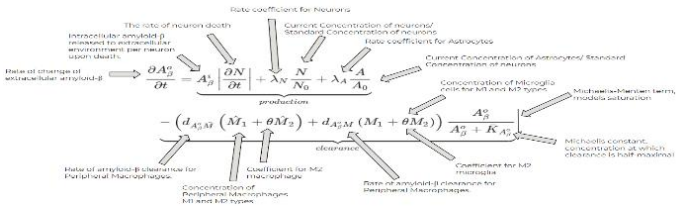
Multiphoton microscopy (MPM) is an advanced imaging technique that enables high-resolution, deep-tissue visualization of living cells and structures, with minimal damage. It works by using near-infrared lasers to excite fluorophores through the simultaneous absorption of two or more photons, making it ideal for studying cellular dynamics in thick tissues like the brain. It allows researchers to track calcium signaling, structural changes, and interactions between astrocytes and amyloid-beta plaques in real-time within intact neural tissue. This helps uncover the roles of astrocytes in neuroinflammation, amyloid-beta clearance, and synaptic dysfunction, which are critical to understanding AD progression and developing targeted therapies.

B. *Single Cell RNA sequencing*

Single-cell RNA sequencing (scRNA-seq) is a powerful technique that enables the detailed profiling of gene expression in individual cells, providing insights into cellular heterogeneity and function. It uncovers distinct subpopulations of astrocytes and their specific roles in Alzheimer's disease progression, such as neuroinflammation, amyloid-beta clearance, and synaptic dysfunction. This technique can help identify therapeutic targets by linking astrocyte subtypes to molecular pathways implicated in AD.

C. *Mathematical Model*

The equation models extracellular amyloid-β (Aβo) production and clearance dynamics, a hallmark of Alzheimer's disease. It captures the contributions of neurons, astrocytes, and immune cells (microglia and peripheral macrophages), integrating these factors to predict amyloid-β accumulation.



Equation 1, Governing equation for amyloid-beta (Aβo) dynamics, illustrating production by neurons and astrocytes and clearance by microglia and macrophages

The production terms reflect the contributions of neurons and astrocytes to amyloid-β levels. Neurons produce amyloid-β both constitutively and during cell death. The first term represents the amyloid-β released due to neuron death, where higher rates of neuron loss increase production due to the release of intracellular amyloid-β to the extracellular environment. This second term represents the baseline production of amyloid-β by healthy neurons, with more neurons contributing to higher production due to Amyloid precursor protein processing (APP processing). As seen in the third term, Astrocytes also contribute to amyloid-β production where larger astrocyte populations result in increased production due to APP processing. This astrocyte-mediated production becomes more significant under pathological conditions, potentially tipping the balance toward amyloid-β accumulation.

The clearance term incorporates the activity of immune cells like microglia (M1 and M2) and peripheral macrophages ((M_1)ˆ and (M_2)ˆ), as well as the saturation effect of clearance mechanisms. The Michaelis-Menten-like term reflects how clearance efficiency decreases as amyloid-β concentrations rise. The parameter KAβ represents the concentration of amyloid-β at which clearance operates at half its maximum capacity. Smaller values correspond to more efficient clearance systems, while larger values indicate less effective responses. The contributions of pro-inflammatory ((M_1)ˆ and M1) and anti-inflammatory ((M_2)ˆ and M2) immune cells are weighted by their respective terms, pro-inflammatory cells excelling at rapid clearance of dense plaques but risking chronic inflammation, and anti-inflammatory cells providing steady

clearance of soluble amyloid-β and promoting tissue repair.

This equation illustrates the delicate balance between amyloid-β production and clearance. When production exceeds clearance, amyloid-β accumulates, leading to plaque formation and Alzheimer's pathology. Astrocyte concentration significantly impacts this balance, with higher astrocyte levels driving greater production. Similarly, the efficiency of clearance is strongly influenced by the Michaelis constant and the activity of immune cells. A lower KAβ enhances clearance efficiency by preventing saturation, while an optimal balance between M1 and M2 activity can improve amyloid-β regulation.

This equation's main limitation is not considering the Astrocyte role in breaking down extracellular amyloid-β. Astrocytes contribute to amyloid-beta (Aβ) degradation by internalizing and breaking down Aβ through lysosomal activity and the release of enzymes like neprilysin and matrix metalloproteinases (MMPs). Additionally, astrocytes play a key role in signaling microglia to clear extracellular Aβ. They release chemokines and cytokines, such as CCL2 and IL-1α, which recruit and activate microglia. Astrocytes also release ATP and other purinergic signals that enhance microglial phagocytosis, facilitating the removal of Aβ plaques and maintaining brain homeostasis

IV. RESULTS

A. *Calcium Signaling and Structural Changes in Astrocytes (Multiphoton Microscopy)*

Results

Using multiphoton microscopy, astrocytes in proximity to Aβ plaques exhibited significantly increased calcium signaling relative to those distally located or outside of plaque regions. As you can see in Figure 2, "Impact of Plaque Proximity on Astrocyte Calcium Activity," the highest levels of calcium activity occurred proximal to high-density plaques and abruptly peaked within 25 µm of the plaque, which decreased with further increased distance from the plaque. Astrocytes within control regions of plaque-free animals maintained low basal calcium activity.

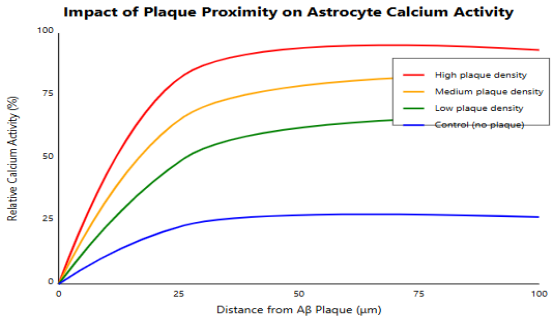


Figure 2, Astrocytic calcium activity versus distance from Aβ plaques, showing varying plaque density conditions (n=15).

In addition to altered calcium signaling, astrocytic hypertrophy was revealed alongside plaques by structural analysis. Indeed, these astrocytes showed enlarged somas, thickened processes, and increased expression of GFAP, a marker of reactive gliosis. The changes were most pronounced in areas surrounding dense-core plaques, suggesting a localized response to the high amyloid load [5, 8, 28].

Discussion

The increased calcium activity in astrocytes near Aβ plaques points to a disturbance in calcium homeostasis, one of the hallmarks of Alzheimer's disease. As you can see in Figure 2, it illustrates a spatial relationship between plaque density and astrocytic calcium signaling: the closer the astrocytes are to high-density plaques, the higher their calcium activity. Astrocytes farther from plaques show less activity, while those in plaque-free regions maintain normal calcium levels. This thus infers that amyloid plaques have a direct and localized effect on astrocyte function [1, 4, 9].

The structural changes, including hypertrophy and increased GFAP levels, indicate that astrocytes around plaques acquire a reactive phenotype. While such a response might be an attempt to reduce Aβ-induced toxicity, it can also perpetuate inflammation due to the release of pro-inflammatory molecules like TNF-α and IL-1β [9, 12], thus being harmful for disease development.

MPM delivered real-time insights into astrocytic responses, thus clearly allowing the visualization of how proximity to plaques influences both calcium dynamics and structural changes. These data emphasize the need for therapies targeting astrocytic calcium dysregulation and ensuing neuroinflammation. As you can see in Figure 2, it emphasizes that investigations into Alzheimer's pathology and subsequent intervention should

focus on astrocytes proximal to high-density plaques [7, 10, 12].

B. Heterogeneous Astrocyte Subtypes and Their Functional Roles (Single-Cell RNA Sequencing)

Results

Single-cell RNA sequencing revealed three astrocyte subpopulations in AD, each displaying different gene expression profiles and functional roles. Pro-inflammatory astrocytes showed upregulation of TNF-α, IL-6, and CCL2, indicating a neuroinflammatory role. These astrocytes clustered around plaques and were associated with markers of oxidative stress that contribute to synaptic dysfunction and neuronal death. Amyloid-clearing astrocytes expressed neprilysin and matrix metalloproteinases, colocalizing with active amyloid-beta (Aβ) clearance and plaque removal both inside and outside of plaque regions. Metabolically dysfunctional astrocytes showed impaired energy metabolism and tau-related signaling, functionally implicating them in mitochondrial dysfunction and tauopathy. Spatial mapping confirmed pro-inflammatory astrocytes were surrounding plaques, amyloid-clearing astrocytes were multispatial, and metabolically dysfunctional astrocytes were localized to areas of neurodegeneration [2, 3, 25].

The following figure gives the effect of the concentration of astrocytes on the dynamics of Aβ. Indeed, increasing concentrations of astrocytes accelerated the reduction rate of Aβ, $\Delta A\beta/\Delta t$, thereby suggesting their active involvement in Aβ homeostasis. The equilibrium line, $\Delta A\beta/\Delta t = 0$, represents a balance between production and clearance of Aβ. The plateau effect, at higher concentrations, for example, 200 astrocytes, is likely because of metabolic or enzymatic limitations.

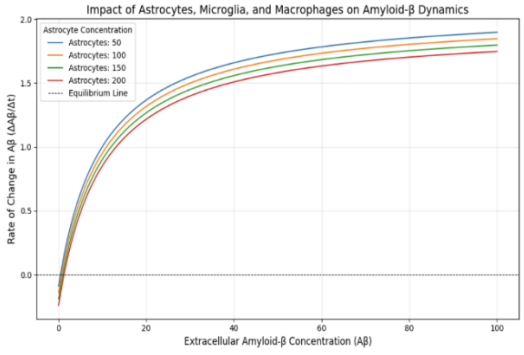


Figure 3, Impact of astrocyte concentration (A) on amyloid-beta dynamics, showing increased production with higher astrocytic activity.

Discussion

Heterogeneous astrocytes reveal protective and pathological contributions in AD. Pro-inflammatory astrocytes perpetuate neuroinflammation via cytokine release, including TNF-α and IL-6, together with oxidative stress, further promoting neuronal death. Amyloid-clearing astrocytes contribute to reduced Aβ burden, facilitating synaptic recovery and neuronal survival, whereas metabolically dysfunctional astrocytes couple impaired energy metabolism with tau propagation and mitochondrial dysfunction in driving neurodegeneration. These findings point to spatially distinct yet complementary contributions by the various subpopulations of astrocytes [12, 23, 25].

This is further supported by the figure, which shows that increasing astrocyte concentrations enhance Aβ clearance but reach a plateau at higher levels, reflecting potential constraints imposed by enzymatic capacity or metabolic dysfunction. Pro-inflammatory astrocytes may inhibit the efficiency of clearance, whereas amyloid-clearing astrocytes drive the positive trends. Dysfunctional astrocytes could reduce overall clearance capacity.

The resolution afforded by scRNA-seq now enables the precise characterization of astrocyte subtypes and opens new therapeutic avenues. Enhancing amyloid-clearing astrocytes through lysosomal activation could accelerate Aβ removal, while inhibiting pro-inflammatory pathways and targeting metabolic dysfunction may mitigate neurodegeneration. These results highlight astrocyte subtypes as distinct therapeutic targets and advance disease-modifying strategies for AD [14, 20, 25].

C. Parameter Impact on Amyloid-Beta Production and Clearance Dynamics (Governing Equation Analysis)

Results

Astrocytes influence Aβ dynamics both by enhancing plaque deposition and clearance, through APP processing, enzymatic degradation, and interactions with microglia. Figure 4 shows that a decrease in KKβ significantly enhances Aβ clearance, suggesting that it is an important therapeutic target. A further support for clearance is provided by the balance of microglial activity

between M1-M2 phenotypes, represented by θ [5, 32, 34].

The governing equation provides a framework for evaluating strategies to alter Aβ dynamics. Decreases in astrocytic production, λA, and/or enhancing the efficiency of clearance, via reductions in KAβ, serve to lower Aβ levels, as illustrated in Figure 4. Simulations reveal that increasing astrocyte concentration, A, increases Aβ production, while decreasing KAβ decreases Aβ accumulation by 25%. Balancing M1/M2 microglial activity decreases Aβ by ~35%, underscoring the need for targeting astrocytic and microglial pathways in Alzheimer's therapies.

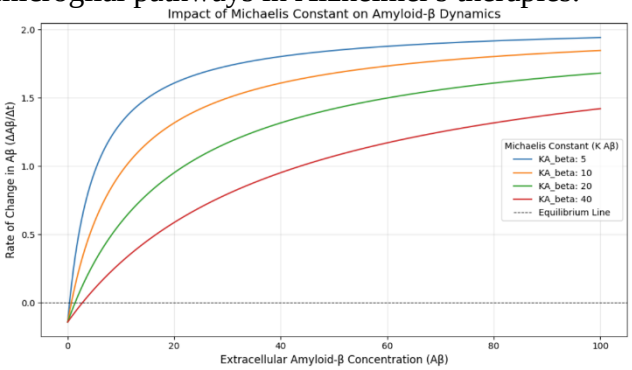


Figure 4, Demonstrates the sensitivity of Aβ clearance efficiency to the Michaelis constant (KAβ), emphasizing the importance of maintaining effective glial activation states.

Discussion

These findings illustrate the dual role of astrocytes in Aβ dynamics. Generally, while astrocytic APP processing contributes to increased plaque deposition, they promote Aβ clearance through their enzymatic degradation pathways and via their engagement with microglia. Model sensitivity to KKβ, as shown in Figure 5, emphasizes its therapeutic utility. Varying KKβ in the model shows that increasing the efficiency of clearance through a pharmacological agent or gene editing of astrocytic pathways has the potential to substantially reduce plaque burden. Sensitivity analysis reveals that even small reductions in KKβ substantially augment Aβ clearance, making it an attractive target for therapy. Furthermore, the modulation of microglial activity to an optimal balance between M1 and M2 phenotypes, represented by θ in the governing equation, may enhance Aβ clearance by shifting microglial behavior toward anti-inflammatory states [5,32,34]

$$\lambda_A \frac{A}{A_0} \quad \text{(Astrocyte Production Term)}$$

$$-\frac{A_\beta^o}{A_\beta^o + K_{A\beta}} \quad \text{(Michaelis-Menten Clearance Term)}$$

$$\theta M_2 \quad \text{(Anti-inflammatory M2 Microglia Contribution)}$$

Figure 5, Key terms from the governing equation showing astrocyte production, clearance efficiency, and microglial contribution to amyloid-beta dynamics.

The governing equation does indeed provide a robust quantitative structure for assessing the impact of therapeutic strategies that modify production and clearance pathways for Aβ. Future iterations of the model might incorporate the mechanisms of astrocytic degradation in order to further improve the predictive accuracy of the model, thus helping the design of AD personalized treatment strategies. It is also possible to analyze changes in astrocytic production, represented by λA, in Figure 5 to see how altering astrocyte populations impacts Aβ clearance and plaque dynamics. This would further emphasize the dichotomy of astrocytes and immune cells in being neuroprotective in certain instances but also neurotoxic under other conditions and underscore the importance of a balance in designing these therapies [13, 33].

V. Conclusion

Our research on the activity of astrocytes in Alzheimer's disease demonstrates their critical role in both brain function and the advancement of illness. These adaptable cells preserve vital brain processes such as synapse maintenance, neurotransmitter control, and blood-brain barrier integrity. The intricate dynamics of reactive astrocytes during the development of AD, especially their dual character as both protective and possibly destructive agents, are discussed thanks to cutting-edge research techniques like single-cell RNA sequencing and multiphoton imaging. Disease mechanisms have been further explored by the discovery of separate A1 (harmful) and A2 (helpful) astrocyte subtypes. Notably, astrocyte calcium dysregulation becomes a major target for treatment, and the Piezo1 channel exhibits promise in regulating inflammation and the healing process. Our understanding of these complex biological interactions has improved thanks to mathematical modeling, which raises the possibility that a multimodal strategy focusing on both astrocytic and neuronal pathways may be necessary

for effective AD treatments. With combination medicines that address both neuronal and astrocytic dysfunction, this thorough understanding of astrocyte biology opens up new therapeutic pathways that could revolutionize AD treatment tactics.

REFERENCES

[1] Nanclares C, Baraibar AM, Araque A, Kofuji P. Dysregulation of Astrocyte–Neuronal Communication in Alzheimer's Disease. Int J Mol Sci. 2021;22(15):7887.

[2] Matias I, Morgado J, Gomes FCA. Astrocyte Heterogeneity: Impact to Brain Aging and Disease. Front Aging Neurosci. 2019;11.

[3] Li K, Li J, Zheng J, Qin S. Reactive Astrocytes in Neurodegenerative Diseases. Aging Dis. 2019;10(3):664-675.

[4] Sofroniew M V. Astrocyte Reactivity: Subtypes, States, and Functions in CNS Innate Immunity. Trends Immunol. 2020;41(9):758-770.

[5] Escartin C, Galea E, Lakatos A, O'Callaghan JP, Petzold GC, Serrano-Pozo A, Steinhäuser C, Volterra A, Carmignoto G, Agarwal A, Allen NJ. Reactive astrocyte nomenclature, definitions, and future directions. Nature neuroscience. 2021 Mar;24(3):312-25.

[6] Escartin C, et al. Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci. 2021;24(3):312-325.

[7] Nanclares C, Baraibar AM, Araque A, Kofuji P. Dysregulation of Astrocyte–Neuronal Communication in Alzheimer's Disease. Int J Mol Sci. 2021;22(15):7887.

[8] Chun H, Lee CJ. Reactive astrocytes in Alzheimer's disease: A double-edged sword. Neurosci Res. 2018;126:44-52.

[9] Pike CJ, Cummings BJ, Cotman CW. Early association of reactive astrocytes with senile plaques in Alzheimer's disease. Exp Neurol. 1995;132(2):172-179.

[10] Nanclares C, Baraibar AM, Araque A, Kofuji P. Dysregulation of Astrocyte–Neuronal Communication in Alzheimer's Disease. Int J Mol Sci. 2021;22(15):7887.

[11] Perez-Nievas BG, Serrano-Pozo A. Deciphering the Astrocyte Reaction in Alzheimer's Disease. Front Aging Neurosci. 2018;10.

[12] Arranz AM, De Strooper B. The role of astroglia in Alzheimer's disease: pathophysiology and clinical implications. Lancet Neurol. 2019;18(4):406-414.

[13] Jha MK, et al. Microglia-Astrocyte Crosstalk: An Intimate Molecular Conversation. Neuroscientist. 2019;25(3):227-240.

[14] Bernardinelli Y, Muller D, Nikonenko I. Astrocyte-synapse structural plasticity. Neural plasticity. 2014;2014(1):232105.

[15] Silburt J, Aubert I. MORPHIOUS: an unsupervised machine learning workflow to detect the activation of microglia and astrocytes. Journal of Neuroinflammation. 2022 Jan 29;19(1):24.

[16] Khakh, B. S. & Sofroniew, M. V. Diversity of astrocyte functions and phenotypes in neural circuits. Nat. Neurosci. 18, 942–952 (2015).

[17] Khakh, B. S. Astrocyte–neuron interactions in the striatum: insights on identity, form, and function. Trends Neurosci. 42, 617–630 (2019).

[18] Allen, N. J. & Barres, B. A. Neuroscience: glia — more than just brain glue. Nature 457, 675–677 (2009).

[19] Allen, N. J. & Lyons, D. A. Glia as architects of central nervous system formation and function. Sci. 362, 181–185 (2018).

[20] Guttenplan K. A., et al., Knockout of reactive astrocyte activating factors slows disease progression in an ALS mouse model. Nat. Commun. 11, 3753 (2020).

[21] Yun S. P., et al., Block of A1 astrocyte conversion by microglia is neuroprotective in models of Parkinson's disease. Nat. Med. 24, 931–938 (2018).

[22] Park J. S., et al., Blocking microglial activation of reactive astrocytes is neuroprotective in models of Alzheimer's disease. Acta Neuropathol. Commun. 9, 78 (2021).

[23] de la Monte SM (2017) Insulin resistance and neurodegeneration: progress towards the development of new therapeutics for Alzheimer's disease. Drugs 77(1):47–65

[24] Raider K, Ma D, Harris JL, Fuentes I, Rogers RS, Wheatley JL, Geiger PC, Yeh HW, Choi IY, Brooks WM et al (2016) A high fat diet alters metabolic and bioenergetic function in the brain: a magnetic resonance spectroscopy study. Neurochem Int 97:172–180

[25] Cai Z, Wan CQ, Liu Z. Astrocyte and Alzheimer's disease. Journal of neurology. 2017 Oct;264:2068-74.

[26] Sailasuta N, Harris K, Tran T, Ross B (2011) Minimally invasive biomarker confirms glial activation present in Alzheimer's disease: a preliminary study. Neuropsychiatr Dis Treat 7:495–499

[27] Morley JE, Armbrecht HJ, Farr SA, Kumar VB (2012) The senescence accelerated mouse (SAMP8) as a model for oxidative stress and Alzheimer's disease. Biochim Biophys Acta 1822(5):650–656

[28] Merlini M, Meyer EP, Ulmann-Schuler A, Nitsch RM (2011) Vascular beta-amyloid and early astrocyte alterations impair cerebrovascular function and cerebral metabolism in transgenic arcAbeta mice. Acta Neuropathol 122(3):293–311

[29] Jhala SS, Hazell AS (2011) Modeling neurodegenerative disease pathophysiology in thiamine deficiency: consequences of impaired oxidative metabolism. Neurochem Int

[30] Escartin C, et al. Reactive astrocyte nomenclature, definitions, and future directions. Nat Neurosci. 2021;24(3):312-325.

[31] Keren-Shaul H, et al. A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. Cell. 2017;169(7):1276-1290.e17.

[32] Arbel-Ornath M, et al. Soluble oligomeric amyloid-β induces calcium dyshomeostasis that precedes synapse loss in the living mouse brain. Mol Neurodegener. 2017;12(1):27.

[33] Martins IC, et al. Mathematical model on Alzheimer's disease. BMC Syst Biol. 2016;10(1):108.

[34] Velasco-Estevez M, et al. Piezo1 regulates calcium oscillations and cytokine release from astrocytes. Glia. 2020;68(1):145-160.

[35] Li, S., & Selkoe, D. J. (2020). A mechanistic hypothesis for the impairment of synaptic plasticity by soluble Aβ oligomers from Alzheimer's brain. Journal of Neurochemistry, 154(6), 583–597. https://doi.org/10.1111/jnc.15007

[36] Danysz, W., & Parsons, C. G. (2012). Alzheimer's disease, β-amyloid, glutamate, NMDA receptors and memantine – searching for the connections. British Journal of Pharmacology, 167(2), 324–352. https://doi.org/10.1111/j.1476-5381.2012.02057.x

[37] Fani, G., La Torre, C. E., Cascella, R., Cecchi, C., Vendruscolo, M., & Chiti, F. (2022). Misfolded protein oligomers induce an increase of intracellular Ca2+ causing an escalation of reactive oxidative species. Cellular and Molecular Life Sciences, 79(9). https://doi.org/10.1007/s00018-022-04513-w

[38] Chen, Y., & Yu, Y. (2023). Tau and neuroinflammation in Alzheimer's disease: interplay mechanisms and clinical

translation. Journal of Neuroinflammation, 20(1). https://doi.org/10.1186/s12974-023-02853-3

[39] First detailed 3D image of astrocytes produced by researchers. Drug Target Review. (2019, October 30). https://www.drugtargetreview.com/news/51509/first-detailed-3d-image-of-astrocytes-produced-by-researchers/

[40] Enger, R., Sprengel, R., Nagelhus, E. A., & Tang, W. (2019). Multiphoton ca2+ imaging of astrocytes with genetically encoded indicators delivered by a viral approach. Neuromethods, 251–277. https://doi.org/10.1007/978-1-4939-9702-2_11

[41] Huang, H., Long, Z., Deng, Y., Huang, Z., Lv, Z., Sun, Q., Liu, H., Liang, H., & Hu, F. (2024). Alterations in astrocyte subpopulations in glioma and identification of cuproptosis-related genes using single-cell RNA sequencing. Journal of Inflammation Research, Volume 17, 6329–6344. https://doi.org/10.2147/jir.s473932

APPENDIX

MatLAB Code to generate results graphs using Equation1, in Methods

```
import numpy as np
import matplotlib.pyplot as plt

# Define fixed parameters for the equation
dA0_microglia = 0.05  # Clearance coefficient for microglia
dA0_macrophages = 0.03  # Clearance coefficient for macrophages
M1_microglia = 2  # Pro-inflammatory microglia concentration
M2_microglia = 1.5  # Anti-inflammatory microglia concentration
theta_microglia = 0.8  # Weighting factor for M2 microglia
M1_macrophages = 1.5  # Pro-inflammatory macrophage concentration
M2_macrophages = 1.2  # Anti-inflammatory macrophage concentration
theta_macrophages = 0.7  # Weighting factor for M2 macrophages
lambda_N = 0.05  # Neuron-driven production coefficient
lambda_A = 0.1  # Astrocyte-driven production coefficient
N = 80  # Current neuron concentration
N0 = 100  # Reference neuron concentration
A0_ref = 100  # Reference astrocyte concentration
A = 100  # Fixed astrocyte concentration
A_beta = np.linspace(0, 100, 500)  # Range of extracellular Aβ concentrations

# Define Michaelis constants to test
KA_beta_values = [5, 10, 20, 40]  # Different values for Michaelis constant

# Plot the graph
plt.figure(figsize=(10, 6))
for KA_beta in KA_beta_values:
    # Production terms
    neuron_production = lambda_N * (N / N0)
    astrocyte_production = lambda_A * (A / A0_ref)
    total_production = neuron_production + astrocyte_production

    # Clearance terms (microglia + peripheral macrophages)
    microglial_clearance = (dA0_microglia * M1_microglia + theta_microglia * M2_microglia) * (A_beta / (A_beta + KA_beta))
    macrophage_clearance = (dA0_macrophages * M1_macrophages + theta_macrophages * M2_macrophages) * (A_beta / (A_beta + KA_beta))
    total_clearance = microglial_clearance + macrophage_clearance

    # Net rate of change in Aβ
    total_change = total_clearance - total_production

    # Plot the results
    plt.plot(A_beta, total_change, label=f'KA_beta: {KA_beta}')

# Customize the plot
plt.title("Impact of Michaelis Constant on Amyloid-β Dynamics", fontsize=14)
plt.xlabel("Extracellular Amyloid-β Concentration (Aβ)", fontsize=12)
plt.ylabel("Rate of Change in Aβ (ΔAβ/Δt)", fontsize=12)
plt.axhline(0, color='black', linestyle='--', linewidth=0.8, label="Equilibrium Line")
plt.legend(title="Michaelis Constant (K Aβ)")
plt.grid(alpha=0.3)
plt.tight_layout()

# Show the plot
plt.show()
```

```python
import numpy as np
import matplotlib.pyplot as plt

# Define parameters for the equation
KA_beta = 10  # Michaelis constant for Aβ clearance
dA0_microglia = 0.05  # Clearance coefficient for microglia
dA0_macrophages = 0.03  # Clearance coefficient for macrophages
M1_microglia = 2  # Pro-inflammatory microglia concentration
M2_microglia = 1.5  # Anti-inflammatory microglia concentration
theta_microglia = 0.8  # Weighting factor for M2 microglia
M1_macrophages = 1.5  # Pro-inflammatory macrophage concentration
M2_macrophages = 1.2  # Anti-inflammatory macrophage concentration
theta_macrophages = 0.7  # Weighting factor for M2 macrophages
lambda_N = 0.05  # Neuron-driven production coefficient
lambda_A = 0.1  # Astrocyte-driven production coefficient
N = 80  # Current neuron concentration
N0 = 100  # Reference neuron concentration
A0_ref = 100  # Reference astrocyte concentration
A_beta = np.linspace(0, 100, 500)  # Range of extracellular Aβ concentrations
astrocyte_concentrations = [50, 100, 150, 200]  # Astrocyte concentrations to test

# Plot the graph
plt.figure(figsize=(10, 6))
for A in astrocyte_concentrations:
    # Production terms
    neuron_production = lambda_N * (N / N0)
    astrocyte_production = lambda_A * (A / A0_ref)
    total_production = neuron_production + astrocyte_production

    # Clearance terms (microglia + peripheral macrophages)
    microglial_clearance = (dA0_microglia * M1_microglia + theta_microglia * M2_microglia) * (A_beta / (A_beta + KA_beta))
    macrophage_clearance = (dA0_macrophages * M1_macrophages + theta_macrophages * M2_macrophages) * (A_beta / (A_beta + KA_beta))
    total_clearance = microglial_clearance + macrophage_clearance

    # Net rate of change in Aβ
    total_change = total_clearance - total_production

    # Plot the results
    plt.plot(A_beta, total_change, label=f'Astrocytes: {A}')

# Customize the plot
plt.title("Impact of Astrocytes, Microglia, and Macrophages on Amyloid-β Dynamics", fontsize=14)
plt.xlabel("Extracellular Amyloid-β Concentration (Aβ)", fontsize=12)
plt.ylabel("Rate of Change in Aβ (ΔAβ/Δt)", fontsize=12)
plt.axhline(0, color='black', linestyle='--', linewidth=0.8, label="Equilibrium Line")
plt.legend(title="Astrocyte Concentration")
plt.grid(alpha=0.3)
plt.tight_layout()

# Show the plot
plt.show()
```