

Modulation of the excitation/inhibition balance by astrocytes in a tripartite synapse model of Alzheimer's disease

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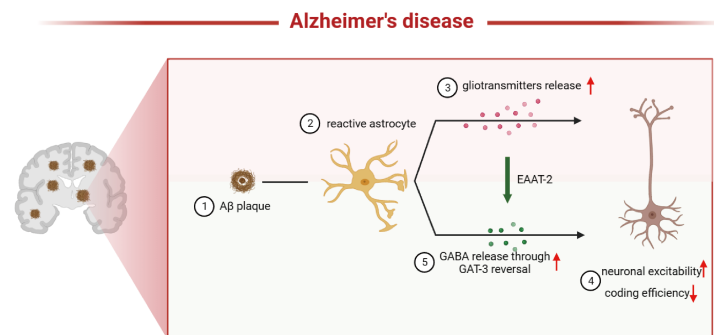
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Graphical Abstract

Astrocytic modulation of glutamate and gamma-aminobutyric acid dysregulates neuronal excitation/inhibition balance in early Alzheimer's disease



Abstract

Alzheimer's disease is a formidable health challenge due to lack of effective therapeutic modalities. The excitation/inhibition imbalance in the early stage of Alzheimer's disease can be potentially considered as a central link between structural brain pathology and cognitive dysfunction. However, the role and effects of reactive astrocytes in the neuronal excitability in early Alzheimer's disease remain unclear. Here, we present a tripartite synaptic model integrating the interactions between neurons and astrocytes than can clarify the role of astrocytes in the regulation of excitation/inhibition. Our model integrates the cation channel transient receptor potential ankyrin 1, whose activation triggers calcium influx, thereby enhancing the fidelity of astrocyte calcium dynamics. Constrained by physiological data, we demonstrate that amyloid- β can activate astrocytes to release glial neurotransmitters, thereby mediating the hyperexcitability of nearby neurons. We also investigate the astrocyte-mediated symbiosis of two neurotransmitters, glutamate and gamma-aminobutyric acid, at the glutamatergic synapse in the context of Alzheimer's disease, to predict the inhibitory compensatory response to excitotoxicity. During excitotoxicity, astrocytes can use the coupling of the excitatory amino acid transporter and gamma-aminobutyric acid transporter to control the concentration ratio of glutamate and gamma-aminobutyric acid in the synaptic cleft, and may convert both through the intracellular gamma-aminobutyric acid synthesis pathway. Our findings reveal that the coding efficiency of neurons diminished as the effects of amyloid- β deepened, establishing a direct link between the pathological features of Alzheimer's disease and cognitive dysfunction. These simulations suggest that astrocytes play a critical role in regulating the neuronal excitation/inhibition balance in the early stage of Alzheimer's disease, thereby influencing the subsequent processes of information transmission, learning, and memory. The pathways characterized by our model present potential therapeutic targets for Alzheimer's disease.

Key Words: Alzheimer's disease; amyloid- β ; astrocyte; computation model; excitation/inhibition; gamma-aminobutyric acid; information transmission; therapeutic target; transient receptor potential ankyrin 1; tripartite synapse

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that causes substantial structural and functional damage to the healthy brain and is primarily characterized by cognitive impairment, memory loss, and behavioral changes (Tenchov et al., 2024). The main pathological features of AD are the accumulation of amyloid- β (A β) and the formation of neurofibrillary tangles. However, no conclusive link has been established between these observations and cognitive symptoms. The inability to explicitly connect biomarkers to progressive memory loss greatly impedes the identification of effective therapeutic interventions for AD (Canter et al., 2016). In particular, despite substantial evidence to support A β -driven neurodegeneration, drugs targeting A β have proven incapable of reversing memory deficits or preventing cognitive decline (Cummings et al., 2023). Since AD is not a biochemical or molecular problem but a physiological one of disrupted cellular connectivity, studies on AD should focus on the complex cellular reactions underlying the long prodromal phase of AD (De Strooper and Karran, 2016; Kurkinen et al., 2023). A thorough understanding of the contributions of these alterations to the progressive dysfunction of brain networks and circuits can help link direct observations in cells to clinically observed cognitive impairments.

Proper brain function requires balanced excitation and inhibition in synaptic transmission through regulation of neuronal excitability. Thus, disruption of the neuronal excitation/inhibition (E/I) balance can lead to dysfunction of brain circuits, causing cognitive impairment. Experimental evidence has shown notable E/I imbalance in the early stages of AD (Bi et al., 2020). Soluble A β , which is highly enriched around amyloid plaques, can mediate neuronal overactivity and lead to neuronal death caused by excitotoxicity (Šišková et al., 2014; Xu et al., 2015). At the neuronal synapse level, A β oligomers have been shown to inhibit long-term potentiation and facilitate long-term depression (Taylor et al., 2021). In addition, soluble tau promotes the silencing of neuronal activity (Šišková et al., 2014). The dual characteristic of increased and decreased excitability not only affects the health of individual neurons, but also affects the normal functional communication between different brain circuits. Thus, E/I imbalance may be an important driving factor of AD-related neurodegeneration and network dysfunction (Maestu et al., 2021).

Astrocytes have been recently recognized as critical mediators of E/I balance in higher-order brain functions such as learning and memory, attention, and plasticity (Chen et al., 2021; Escalada et al., 2024). In fact, the potential involvement of astrocytes in the integration of neural activity and E/I regulation and their influence on behavior have

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been widely discussed (Yu et al., 2020; Nagai et al., 2021; Liu et al., 2022). Astrocyte processes envelop the synaptic cleft and form tripartite synapses through close interaction with presynaptic and postsynaptic neurons. These processes sense synaptic activity through metabolic glutamate receptors (mGluRs) on the membrane and release gliotransmitters that act on presynaptic or postsynaptic neurons to modulate excitatory and inhibitory activity (Liu et al., 2021). In addition, they express excitatory amino acid transporter (EAAT-2), gamma-aminobutyric acid (GABA) transporter (GAT-3), and other transporters to regulate synaptic uptake of glutamate and GABA, ensure the normal neurotransmitter concentration in the synaptic cleft, and maintain the E/I balance of the brain, which is essential for neuronal activity and central nervous system homeostasis (Sood et al., 2021). In AD, normal astrocytes transform into abnormal reactive astrocytes, which undergo morphological, molecular, and functional remodeling (Escartin et al., 2021). Many studies have identified the presence of reactive astrocytes in patients with AD and mouse models, especially around Aβ plaques (Heneka et al., 2005; Carter et al., 2012; Chun and Lee, 2018). Reactive astrocytes have even been detected in the early stages of AD, before neuronal death, and are prevalent throughout disease progression (Perez-Nievas and Serrano-Pozo, 2018). Reactive astrocytes near the plaques show abnormal calcium dynamics (Agulhon et al., 2012), which are characterized by higher-frequency and longer-duration calcium waves (Huffels et al., 2022). Additionally, increased Ca²⁺-dependent glutamate release has been shown to promote neuronal excitotoxicity (Agulhon et al., 2012). On the other hand, the expression of EAAT-2 and GAT-3 on the membrane is also altered (Huffels et al., 2023), while the intracellular synthesis of GABA and glutamate is elevated (Andersen et al., 2022; Tang et al., 2023). Consequently, the dysregulated release of glutamate and GABA by astrocytes in the context of AD regulate the E/I balance of nearby neurons at multiple nodes, limiting the ability to comprehensively evaluate the influence of multiple factors on E/I imbalance and the importance of each node separately in AD.

The transient receptor potential ankyrin 1 (TRPA1) channel, a stress-sensing cation channel, plays an important role in excitability changes in neurons and astrocytes. TRPA1-mediated resting calcium levels in astrocytes also influence the expression of GAT-3 on the plasma membrane, thereby affecting the GABA concentration in the synaptic cleft and nearby neuronal firing activity (Shigetomi et al., 2011). Thus, alterations in TRPA1-mediated calcium influx significantly affect astrocytic calcium oscillations and communication with neurons, influencing excitability in both presynaptic and postsynaptic neurons. In the context of AD, experiments conducted on mice have demonstrated that TRPA1 channels are involved in excessive activity in early-stage neurons and astrocytes. The Aβ-induced increase in intracellular Ca²⁺ levels in astrocytes is TRPA1-dependent (Lee et al., 2016). Recent studies have indicated that blocking TRPA1 normalizes astrocytic activity, prevents the withdrawal of perisynaptic astrocytic processes, protects against neuronal dysfunction, and maintains synaptic structural integrity. Long-term blockade can also reduce Aβ deposition and enhance spatial memory (Payrits et al., 2020; Paumier et al., 2022). Thus, the toxic effects of Aβ triggered by TRPA1 channel activation are crucial for the progression of AD, and the TRPA1 channel holds promise as a potential therapeutic target for promoting neuroprotection.

Experimental studies have shown how astrocytes regulate the E/I balance of neurons, focusing on some specific aspects of this complex problem (Agulhon et al., 2012; Aldabbagh et al., 2022; Paumier et al., 2022). However, research that synthesizes multiple experimental results and places them in the context of AD is lacking, limiting the ability to link the roles of Aβ in the AD hypothesis with the functions of astrocytes and the regulation of E/I balance. Considering the complex and diverse roles of astrocytes in the regulation of E/I balance, we aimed to integrate multiple mechanisms of neurons and astrocytes to better understand the role of astrocytes in the development of AD by participating in the E/I balance. This research is expected to deepen the existing understanding of nervous system function and neurodegenerative diseases, and establish more accurate pathological models to explore useful treatment methods. Among gliotransmitters, GABA and glutamate are metabolically interconnected and exert opposing effects on neuronal firing. Experimental findings have shown that reactive astrocytes excessively release glutamate into the extracellular space, affecting both presynaptic and postsynaptic neurons and thereby promoting excitation. Furthermore, while astrocytes maintain a certain concentration of glutamate in the synaptic cleft by taking up glutamate through EAAT-2, reactive astrocytes undergo morphological changes and exhibit decreased expression of EAAT-2, which impairs the clearance of synaptic glutamate. Third, astrocytes also release and take up GABA through the bestrophin-1 (Best-1) channel and GAT-3 (Kilb and Kirischuk, 2022). GAT-3 mainly takes up GABA to clear synaptic GABA in physiological conditions. However, under pathological conditions, increased synthesis of GABA in reactive astrocytes (Jo et al., 2014) and elevated synaptic glutamate concentrations reverse the direction of the GAT-3 transporter from uptake to release. The enhanced tonic inhibition potentially mitigates excitotoxic damage (Aldabbagh et al., 2022), but also inhibits long-term potentiation, causing learning and memory deficits (Wu et al., 2014). Moreover, EAAT-2 and GAT-3 are coupled and highly dependent on changes in Na⁺ concentration gradients across the membrane (Héja et al., 2012). They are also influenced by the intracellular and extracellular concentrations of glutamate and GABA, adding complexity to studies of synaptic glutamate and GABA. These factors clearly indicate that in AD, disturbances in astrocytic uptake, synthesis, and the metabolism of glutamate and GABA counteract the E/I balance, resulting in abnormal synaptic function.

Encoding of neural information, wherein the brain utilizes certain features of action potential sequences (spike trains) to encode sensory and cognitive information, is one

of the core topics in neuroscience. The balance between the excitatory and inhibitory synaptic membrane currents received by neurons forms the foundation for their spontaneous firing and responsiveness to sensory input, making E/I balance a primary requirement for efficient encoding (Zhou and Yu, 2018). Computational models have explored how E/I balance promotes coding efficiency and energy efficiency in cortical neurons. For instance, Sengupta et al. (2013) used a single-compartment computational model with stochastic voltage-gated ion channels to calculate the information content under E/I-balanced or E/I-unbalanced conditions. They found that neurons with balanced synaptic current exhibited similar information rates but used fewer, more informative peaks than under unbalanced conditions, resulting in lower energy consumption. Boerlin et al. (2013) demonstrated that highly random net input currents would lead to highly variable neural responses if the excitatory and inhibitory currents offset each other. Variable neural responses and E/I balance are both necessary outcomes for neural networks to effectively represent information in their pulse sequences. Here, we aim to observe how information encoding efficiency changes with increasing Aβ concentrations in our model, thereby elucidating the pathological cascade of E/I imbalance on the development of AD.

Computational modeling, as an essential complementary approach that links together different biochemical and biophysical reactions and process dynamics, is crucial to systematically map progressive cellular alterations and to generate a comprehensive cellular theory of AD. This approach can integrate empirical results into a meaningful common framework rather than summarizing individual findings. In this study, with TRPA1 as an entry point and the accumulation of Aβ as the trigger for AD, we aimed to investigate how astrocytes regulate neuronal excitability by controlling extracellular glutamate and GABA concentrations, culminating in the disruption of the E/I balance. Concurrently, we explored changes in information transmission efficiency during the development of AD and therapeutic interventions for correcting the perturbed E/I equilibrium.

Methods

This computational modeling study used exclusively published parameters, equations, and validation data. We propose a tripartite glutamatergic synaptic model, and simulate all of the above-mentioned mechanisms using biologically validated parameters from the published experimental data. Since the study involved no human/animal subjects, clinical data, or new experimental data, ethical approval and informed consent were not applicable per standard research guidelines for theoretical modeling work. The full synapse model coupled the following key signaling pathways of astrocytes affecting E/I balance: (i) Regulation of presynaptic release and postsynaptic slow inward current mediated by glutamate release from astrocytes; (ii) uptake of glutamate in the synaptic cleft by EAAT-2; and (iii) uptake and release of GABA in the synaptic cleft by GAT-3. Furthermore, the model will account for the coupling relationship between EAAT-2 and GAT-3. The tripartite synaptic model is illustrated in **Figure 1**, and all the variables and parameters are listed in Additional **Table 1**.

Presynaptic dynamics

Presynaptic neurons use the classical Hodgkin–Huxley model to describe the action potential generated by the activation and inactivation of different ion channels on the plasma membrane (Hodgkin and Huxley, 1952):

$$C \frac{dV_{pre}}{dt} = I_{app} - I_{Na} - I_K - I_L \quad (1)$$

where I_{Na} , I_K , I_L , and reflect voltage-gated Na⁺, K⁺, and leaked presynaptic currents, respectively. I_{app} is an applied stimulus with a current density of 5 μA/cm².

To better study the regulation of synaptic glutamate release (Tsodyks et al., 1998) by gliotransmitter-bound extrasynaptic presynaptic receptors, we utilized the previously published synaptic model of glutamatergic gliotransmission by De Pitta and Brunel (2016) to describe the effects of astrocytes on presynaptic neurons in AD:

$$\tau_f \frac{d}{dt} u_s = -u_s + \sum_k u_0 (1 - u_s) \delta(t - t_k) \tau_f \quad (2)$$

$$\tau_d \frac{d}{dt} x_s = 1 - x_s - \sum_k r_s(t) \delta(t - t_k) \tau_d \quad (3)$$

where u_s is the probability of glutamate resources to be available for release; x_s is the readily releasable glutamate resource; and k is the number of action potentials. Each time the action potential reached 20 mV at time t_k , u_s increased by the factor u_0 , while x_s decreased by a portion $r_s = u_s(t_k) \chi_s(t_k)$, corresponding to the fraction of effectively released glutamate. u_0 may be interpreted as the synaptic release probability at rest.

The extracellular glutamate concentration determines the proportion of presynaptic receptors in the extrasynaptic space (De Pitta and Brunel, 2016):

$$\tau_p \frac{d}{dt} \gamma_s = -\gamma_s + O_p (1 - \gamma_s) G_{A\tau_p} \quad (4)$$

where O_p and τ_p , respectively, denote the rise rate and decay time of the effect of gliotransmission on synaptic glutamate release.

Therefore, modulation of synaptic release by gliotransmitter-bound presynaptic receptors

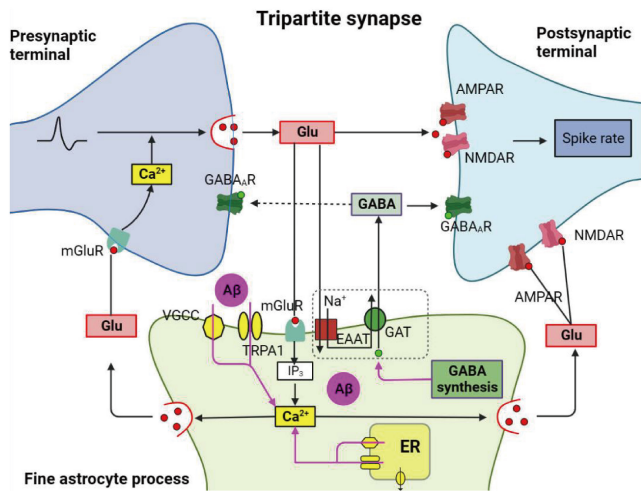


Figure 1 | Schematic diagram of the tripartite synaptic model.

Aβ, which targets the TRPA1 channel, leads to increased Ca²⁺ influx, resulting in abnormally elevated intracellular calcium concentrations within astrocytes. The excessive excitation of astrocytes contributes to the augmented release of glutamate into the extrasynaptic space, resulting in an increase in Ca²⁺ concentration in presynaptic neurons, which in turn affects the release of synaptic glutamate and the excitability of postsynaptic neurons and astrocytes and causes excitotoxicity. Glutamate in the extrasynaptic space can also bind to receptors on the membrane of postsynaptic neurons and affect the membrane potential and calcium activity. Synaptic glutamate can be absorbed by astrocyte membrane-bound EAAT-2, accompanied by synaptic Na⁺ and astrocyte K⁺ transport. The entry of Na⁺ into astrocytes triggers the release of GABA into the synaptic cleft through GAT-3, where it activates inhibitory ionotropic GABA receptors located on presynaptic and postsynaptic neuronal membranes, thereby reducing neuronal excitability. **AMPA: xxx** Aβ: Amyloid-β; EAAT: excitatory amino acid transporter; ER: endoplasmic reticulum; GABA: gamma-aminobutyric acid; GAT: GABA transporter; GAT-3: GABA transporter; mGluR: metabolic glutamate receptor; **NMDAR: xxx** TRPA1: transient receptor potential ankyrin 1; VGCC: voltage-gated calcium channel.

is assumed to be attributable to the modulation of the probability of resting synaptic release (De Pitta and Brunel, 2016):

$$u_0(\gamma_s) \approx U_0 + (\xi - U_0)\gamma_s \quad (5)$$

where U_0 denotes the synaptic release probability at rest in the absence of gliotransmission, whereas the **parameter ξ lumps** in a phenomenological way, the information regarding the effect of gliotransmission on synaptic release. Here, we **considered $U_0 < \xi \leq 1$** , indicating a “release-increasing” effect of astrocytic gliotransmitters on synaptic release.

Synaptic glutamate

The glutamate concentration g in the synaptic cleft is affected by the release of presynaptic neurons and the clearance of astrocytes, as shown below (Tewari and Majumdar, 2012):

$$\frac{dg}{dt} = \sum_k \rho_c Y_i r_s(t_k) \delta(t - t_k) - g_c \cdot g \quad (6)$$

where ρ_c is the volume ratio between glutamate-containing neuronal vesicles and the synaptic cleft, Y_i is the total concentration of glutamate in vesicles, and $r_s(t_k)$ is the fraction of effective released glutamate. g_c is the glutamate clearance rate, that is, the reuptake rate of glutamate by neurons or astrocytes.

Astrocyte dynamics

Ca²⁺ usually enters the cell through active transport of different types of voltage-gated calcium channels (VGCCs) and passive leakage distributed on the membrane. However, in astrocytes, IP₃-dependent calcium-induced calcium release from the endoplasmic reticulum is considered to be the main mechanism for intracellular Ca²⁺ dynamics. In the present study, we added TRPA1-mediated calcium influx on the basis of Liu’s multi-pathway calcium dynamic model (Liu et al., 2021):

$$\frac{dc_{ast}}{dt} = \underbrace{J_{VGCC} + J_{TRPA1}}_{\text{transmembrane fluxes}} - J_{out} + \underbrace{J_{IP_3R} + J_{RyR} + J_{leak} - J_{SERCA}}_{\text{ER exchange}} \quad (7)$$

$$\frac{dc_{ER}}{dt} = (J_{IP_3R} + J_{RyR} + J_{leak} - J_{SERCA})/f_v \quad (8)$$

where J_{VGCC} represents the pathway of Ca²⁺ influx through four types of VGCCs, namely, the L-, N-, T-, and R-types. J_{IP_3R} and J_{RyR} refer to the Ca²⁺ flowing into the cytoplasm

through inositol trisphosphate receptors (IP₃Rs) and ryanodine receptors (RyRs) on the endoplasmic reticulum (ER), respectively. J_{leak} is the leakage flux from the ER. J_{SERCA} represents the sarcoplasmic/endoplasmic reticulum Ca²⁺ ATPase (SERCA) pump of the ER to refill the ER by pumping Ca²⁺ back from the cytoplasm.

J_{TRPA1} is the calcium flux through TRPA1. Using Faraday’s law, the Ca²⁺ flux can be expressed as:

$$J_{TRPA1} = - \frac{I_{TRPA1}}{zFV_{ol_{ast}}} \quad (9)$$

where z is the valence of Ca²⁺, F is the Faraday constant and $V_{ol_{ast}}$ is the volume of astrocytes.

TRPA1 is a thermosensitive and stress-sensing cation channel that can be activated by various environmental stimuli or endogenous molecules involved in neuroinflammation. It may also be triggered through allosteric mechanisms. Here, we used the allosteric kinetic model to clarify how TRPA1 may respond synergistically to different stimuli, such as temperature and agonists. By establishing different sensors (temperature and chemical) in different structural domains of the TRPA1 tetramer, each structural domain is allosterically coupled to the channel gate, thereby influencing the channel open probability (P_o) and the incoming calcium flow. The allosteric model of temperature and agonist concentration is provided by the following equations (Salazar et al., 2011):

$$I_{TRPA1} = N \cdot g_{TRPA1} \cdot P_o \cdot (V_{ast} - V_{rev}) \quad (10)$$

$$P_o = \frac{1}{1 + \frac{1 + K + Q + KQ}{L_o(1 + KC_a + QF + KC_aQF_a)}} \quad (11)$$

$$Q = \frac{[Ligand]}{K_D} \quad (12)$$

$$K = \exp\left(-\frac{\Delta H - T\Delta S}{RT}\right) \quad (13)$$

where N is the number of channels, g_{TRPA1} is the conductance value of TRPA1, and $V_{ast} - V_{rev}$ is the difference between the resting potential of astrocytes and the reversal potential of TRPA1. L is the equilibrium constant between the closed and open states when all sensors are relaxed; K and Q are the constants that govern the temperature- and agonist-dependent transitions; and C and F are the allosteric constants that couple the temperature and agonist sensors to the channel gating, respectively. **[Ligand]** generally refers to the role of agonists that can promote the inflow of TRPA1 calcium under physiological conditions, K_D is the dissociation constant of the agonist, and T is the absolute temperature. ΔS and ΔH represent the difference in entropy and enthalpy between different forms of TRPA1 affected by temperature, respectively.

To show that the presence of glutamate in the synaptic cleft can lead to an increase in astrocyte Ca²⁺ levels through mGluR, we referred to a part of the G-ChI model proposed by De Pittà et al. (2009), wherein the mGluR pathway mediates Ca²⁺ oscillation in astrocytes. Here, glutamate mainly increased the Ca²⁺ levels of the IP₃R channel in the cytoplasm by affecting the production of IP₃ in the PLCβ pathway. Therefore, the Ca²⁺ flux of IP₃R and the dynamics of intracellular IP₃ are as follows:

$$J_{IP_3R} = r_{ca} m_{\infty}^3 n_{\infty}^3 c_{ast}^3 (c_{ast} - c_{ER}) \quad (14)$$

$$\frac{dp_{ast}}{dt} = v_{\beta} \cdot \text{Hill}\left(g^{0.7}, K_R \left(1 + \frac{K_P}{K_R} \text{Hill}(c_{ast}, K_R)\right)\right) + \frac{v_G}{1 + \frac{v_{G_{max}}}{K_G}} \cdot \text{Hill}(c_{ast}^2, K_{PLC\beta}) - v_3 \cdot \text{Hill}(c_{ast}^4, K_{ad}) \text{Hill}(p_{ast}, K_3) - r_{p_{p_{ast}}} \quad (15)$$

$$m_{\infty} = \text{Hill}(p_{ast}, d_1), n_{\infty} = \text{Hill}(c_{ast}, d_5), \text{Hill}(x^n, K) = \frac{x^n}{x^n + K} \quad (16)$$

$$\frac{dh_{ast}}{dt} = \alpha_{h_{ast}}(1 - h_a) - \beta_{h_{ast}} h_{ast} + G_h(t) \quad (17)$$

$$\alpha_{h_{ast}} = a_2 d_2 \frac{p_{ast} + d_1}{p_{ast} + d_3}, \beta_{h_{ast}} = a_2 c_{ast} \quad (18)$$

In equation 14, r_{ca} is the maximum rate of Ca²⁺ flux from IP₃R. The first two terms on the right side of equation 15 denote IP₃ produced by two specific phospholipases PLCβ and PLCδ, respectively, and the last two terms denote the degradation of IP₃ by IP₃-3k and IP₅P, respectively. The g in the first term is the concentration of glutamate in the synaptic cleft, which indicates its effect on the Ca²⁺ oscillation of astrocytes. $G_h(t)$ is a zero-mean, uncorrelated Gaussian white noise term with a covariance function. $\text{Hill}(x^n, K)$ is the generic Hill function.

The model of glutamate secretion by astrocytes is similar to that of presynaptic glutamate release. Assuming that a fraction $\chi_A(t)$ of the gliotransmitter resources is available for release at any time t , then every time t_i that astrocytic Ca²⁺ exceeds a certain threshold concentration, C_{θ} , a fraction of the readily releasable astrocytic glutamate resources, that is, $r_A(t_i) = U_{\chi_A}(t_i)$, is released into the extrasynaptic space. The readily releasable glutamate resources are determined on the basis of the method described by De Pitta and Brunel (2016):

$$\tau_G \frac{d}{dt} \chi_A = 1 - \chi_A - \sum_j r_A(t) \delta(t - t_j) \tau_G \quad (19)$$

Assuming that the clearance rate of glutamate was $1/\tau_c$, the time course of astrocyte-derived glutamate in the extracellular space is given by De Pitta and Brunel (2016):

$$\tau_c \frac{d}{dt} G_A = -G_A + \sum G_{rel}(t) \delta(t - t_i) \tau_c \quad (20)$$

where G_{rel} denotes a quantal glutamate release event by the astrocyte at $t = t_i$, as $G_{rel}(t_i) = \rho_v G_{tr}(t_i)$. G_{tr} represents the total vesicular glutamate concentration in the astrocyte, and ρ_v is the volume ratio between glutamate-containing astrocytic vesicles and the periastrorcytic space.

Amyloid- β hypothesis

In the early stage of AD, reactive astrocytes near A β plaques show abnormal calcium dynamics (Aguilhon et al., 2012). A β has been shown to trigger astrocytes to produce calcium transients and calcium oscillations, and this effect involves a variety of astrocyte cell membrane Ca^{2+} channels and ER Ca^{2+} release (Alberdi et al., 2013). Here, we followed the A β hypothesis in Liu's multi-calcium flow model (Liu et al., 2021), and used the parameter " a " to represent the fixed A β concentration, which corresponds to a certain stage of AD progression. For example, a small value of " a " may reflect a lower level of A β in AD. By changing the concentration of A β , that is, the parameter variable " a ," we can easily simulate the induction of A β on the calcium excitability of astrocytes at different stages of early AD development. More importantly, considering the important role of the TRPA1 channel in the excitatory changes of neurons and astrocytes, we added the modeling of this channel to Liu's multi-calcium flow model (Liu et al., 2021), thereby extending the calcium dynamics model of astrocytes and integrating the complex mechanism of A β in AD to explore the role of A β more comprehensively.

Studies have shown that the A β -induced increase in Ca^{2+} levels in astrocytes is TRPA1-dependent (Lee et al., 2016; Payrits et al., 2020; Paumier et al., 2022). Therefore, we modified the allosteric kinetic model for describing the activation of A β on the calcium flux in TRPA1 channels by changing the concentration of the agonist to increase the probability of channel opening:

$$Q = \frac{[Ligand] + k_{TRPA1}a}{K_D} \quad (21)$$

where k_{TRPA1} controls the intensity of the effect of A β on the channel.

Postsynaptic action potentials

On the membrane of postsynaptic neurons, both N-methyl-D-aspartate acid receptors and α -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors can bind to glutamate in the synaptic cleft and then mediate excitatory neurotransmission and synaptic plasticity. The membrane potential of postsynaptic neurons can be expressed as described in a previous study (Liu et al., 2023):

$$C \frac{dV_{post}}{dt} = -I_{Na} - I_K - I_L - I_{NMDA} - I_{AMPA} - I_{SIC} \quad (22)$$

where I_{SIC} denotes a slow inward current to postsynaptic neurons mediated by glutamate released by astrocytes.

Dynamics of excitatory amino acid transporter and gamma-aminobutyric acid transporter

E/I imbalance is considered to be the basis of diseases such as AD. Glial cells play an important role in maintaining E/I balance and may cause disturbances in this balance. Astrocytes express various transporters to regulate E/I balance, including EAAT-2 and GAT-3 (Chen et al., 2021; Liu et al., 2022). To explore how astrocytes affect neuronal excitability by maintaining the concentration of glutamate and GABA, the two most important neurotransmitters, we combined previously established tripartite synapse model components with novel components (dynamics of EAAT and GAT) developed in this study to create a new synapse model. This part refers to the work of Flanagan et al. (2021).

Since the transport of glutamate by EAAT-2 depends on the concentration gradient of Na^+ and K^+ across the membrane, while the transport of GABA by GAT-3 also depends on the concentration gradient of Na^+ , the changes in the Na^+ concentration gradient couple the two transporters together (Héja et al., 2012). Therefore, we modeled the astrocyte membrane ion current, including the changes in glutamate, K^+ , Na^+ , and GABA concentrations, and used the following equations (Flanagan et al., 2021):

$$I_{Na,ast} = 1.5I_{EAAT} + 3I_{NKA} + 3I_{NCX} + 2I_{GAT} + I_{Na,L} \quad (23)$$

$$I_{K,ast} = -0.5I_{EAAT} + 2I_{NKA} + I_{Kir} + I_{K,L} \quad (24)$$

$$I_{Glu,ast} = -0.5I_{EAAT} + I_{Glu,L} \quad (25)$$

$$I_{GABA,ast} = I_{GAT} \quad (26)$$

where I_{EAAT} , I_{NKA} , I_{NCX} , I_{GAT} and I_{Kir} denote the currents generated by EAAT-2, Na^+/K^+ -ATPase (NKA), Na^+/Ca^{2+} exchanger (NCX), GAT-3, and the inward rectifying K^+ channel, respectively. The leakage currents of Na^+ , K^+ , and glutamate are represented by $I_{Na,L}$, $I_{K,L}$ and $I_{Glu,L}$, respectively, which provide model stability. To study the effects of these currents on the concentration alone, the membrane potential of astrocytes was set to be constant.

The currents generated by EAAT-2, NKA, NCX, GAT-3, and inward rectified K^+ channels are specified as follows (Flanagan et al., 2021):

$$I_{Kir} = g_{Kir} \cdot \sqrt{K_{syn}} \cdot (V_{ast} - E_{Kir}) \quad (27)$$

$$I_{NCX} = I_{NCX,max} \left(\left(\frac{Na_{ast}}{Na_{syn}} \right)^3 e^{\frac{EFV_{ast}}{RT}} - \left(\frac{Ca_{ast}}{Ca_{syn}} \right) e^{\frac{(C-1)FV_{ast}}{RT}} \right) \quad (28)$$

$$I_{NKA} = I_{NKA,max} \left(\frac{Na_{ast}^{1.5}}{Na_{ast}^{1.5} + K_{Na}^{1.5}} \right) \left(\frac{K_{syn}}{K_{syn} + K_{Ke}} \right) \quad (29)$$

$$I_{EAAT} = -\alpha_{EAAT} \cdot e^{-\beta_{EAAT}(V_{ast} - E_{EAAT})} \quad (30)$$

$$E_{EAAT} = \frac{RT}{2F} \ln \left(\left(\frac{Na_{syn}}{Na_{ast}} \right)^3 \left(\frac{H_{syn}}{H_{ast}} \right) \left(\frac{Glu_{syn}}{Glu_{ast}} \right) \left(\frac{K_{syn}}{K_{syn}} \right) \right) \quad (31)$$

$$I_{GAT} = g_{GAT}(V_{ast} - E_{GAT}) \quad (32)$$

$$E_{GAT} = \frac{RT}{F} \ln \left(\left(\frac{Na_{syn}}{Na_{ast}} \right)^2 \left(\frac{GABA_{syn}}{GABA_{ast}} \right) \left(\frac{Cl_{syn}}{Cl_{ast}} \right) \right) \quad (33)$$

where V_{ast} is the resting potential of astrocytes and ε is the NCX partition parameter. For details regarding the parameter values, see Table in the appendix. The starting values of each ion in and out of the cell were set on the basis of the experimental literature and updated at each step of the simulation. The intracellular concentration of calcium ions was calculated using the value of equation 7, and the extracellular concentration was calculated separately considering the previous calcium-induced calcium release model:

$$\frac{dc_{ast}}{dt} = \frac{J_{VGCC} + J_{TRPA1} - J_{out} + I_{NCX} + J_{IP3R} + J_{RyR} + J_{leak} - J_{SERCA}}{\text{transmembrane fluxes} \quad \text{ER exchange}} \quad (34)$$

$$\frac{dc_{syn}}{dt} = -\frac{I_{NCX}S_A}{zFV_{ol_s}} - (J_{TRPA1} - J_{VGCC} - J_{leak}) \frac{Vol_{ast}}{Vol_s} \quad (35)$$

where S_A is the surface area of the peri-synaptic astrocytic membrane, and Vol_{ast} and Vol_s are the volume of astrocyte and synaptic compartments, respectively.

The currents were converted to ionic fluxes by Faraday's law, where the change in the astrocytic concentration of ion X and corresponding change in synaptic concentration are given by Flanagan et al. (2021):

$$\frac{dX_{ast}}{dt} = -\frac{I_{X,ast}S_A}{zFV_{ol_{ast}}}, X = Na^+, K^+, Glu, GABA \quad (36)$$

$$\frac{dX_{syn}}{dt} = -\frac{I_{X,ast}S_A}{zFV_{ol_s}}, X = Na^+, K^+, Glu, GABA \quad (37)$$

The action potentials of presynaptic neurons and postsynaptic neurons were changed to:

$$C \frac{dV_{pre}}{dt} = I_{app} - I_{Na} - I_K - I_L - I_{preGABAA} \quad (38)$$

$$C \frac{dV_{post}}{dt} = -I_{Na} - I_K - I_L - I_{NMDA} - I_{AMPA} - I_{SIC} - I_{postGABAA} \quad (39)$$

where $I_{preGABAA}$ and $I_{postGABAA}$ are GABA $_A$ -mediated currents on presynaptic and postsynaptic membranes, respectively, in response to synaptic GABA released by astrocytes.

Simultaneously, to describe glutamate uptake in the synaptic cleft by EAAT-2, the corresponding equation for glutamate concentration in synaptic cleft was changed to:

$$\tau_c \frac{dg}{dt} = \sum_k \rho_c Y_{tr}(t_k) \delta(t - t_k) \tau_c - \frac{I_{Glu,ast}S_A}{zFV_{ol_s}} \quad (40)$$

Information entropy

The method described by Strong et al. (1998) was used to measure the entropy of the responses, which compares different spike trains without reference to the stimulus parameters. The total entropy sets the information capacity for the spike trains. The noise entropy measures the variability of the spike trains across trials. These quantities are determined by the temporal resolution with which the spikes were sampled, Δt (1 ms), and the size of the time window T_w . We presented different A β concentrations to generate different spike trains in each subsequent trial to calculate the total entropy, and we repeated the trial at the same A β concentration to calculate the noise entropy. We divided the spike trains to form L-letter words ($L = 6, 16, 48, 64$), where $T_w/L = \Delta t$. We also used the responses of the spike trains from different A β concentrations to estimate the probability of occurrence of particular word, $P(L)$. The total entropy is given by:

$$S_{total} = -\sum_L P(L) \log_2 P(L) \quad (41)$$

We kept the A β concentration unchanged to the word probability calculated at different time points in the spike trains, $P(L/t)$, and calculated the entropy to obtain the noise entropy by average addition:

$$S_{noise} = \langle -\sum_L P(L/t) \log_2 P(L/t) \rangle_t \quad (42)$$

The information rate was calculated as:

$$I_{\text{entropy}} = S_{\text{total}} - S_{\text{noise}} \quad (43)$$

The real total entropy and noise entropy could be obtained in the limit $T_w \rightarrow \infty$. Based on the entropy obtained by different lengths, we performed linear extrapolation of the entropy values of the reciprocals of the five lengths on the entropy map to $T_w \rightarrow \infty$ to obtain the real values.

Results

Initial simulations of the glutamatergic synaptic model revealed that elevated A β concentrations induce pathological hyperexcitability in both astrocytes and synaptic neurons. Specifically, A β -TRPA1 channel interactions triggered aberrant astrocytic calcium dynamics, which manifested as increased oscillation frequency and amplitude. This astrocytic hyperactivity potentiated extracellular glutamate release, which enhanced presynaptic release probability and ultimately elevated postsynaptic firing rates.

Extension of the model to incorporate EAAT-2/GAT-3 co-localization and GABAergic signaling demonstrated astrocyte-mediated E/I imbalance under AD conditions. During excitotoxicity, heightened synaptic glutamate concentrations drove sodium-dependent GABA efflux through reversed GAT-3 transport, while intracellular neurotransmitter synthesis further modulated inhibitory compensation. These coordinated alterations reduced the efficiency of neural coding and increased metabolic demand through frequent action potentials, potentially disrupting learning and memory circuits.

Amyloid- β -induced excessive Ca^{2+} activity in astrocytes through transient receptor potential ankyrin 1 channels

In this study, we integrated multiple A β -affected calcium channels into an astrocyte model to simulate the induction of A β on the membrane and intracellular calcium channels at different stages of early AD development. We achieved this by varying the A β concentrations, i.e., the parameter variable " a ," which led to an increase in the frequency of calcium oscillations in astrocytes (Figure 2A). The results indicated that A β significantly disrupts intracellular Ca^{2+} homeostasis. In the presence of A β , astrocytic $[\text{Ca}^{2+}]$ increases, resulting in both increased amplitude and frequency of calcium oscillations. Notably, as the activating effect of A β on TRPA1 channels strengthened, the basal calcium level also intensified.

Previous experiments have shown that TRPA1 influences microdomain calcium signaling and basal calcium levels of astrocytes (Shigetomi et al., 2011), with TRPA1 blockade reducing the basal calcium level in astrocytes from 120 nM to 60 nM (Shigetomi et al., 2011). As shown in Figure 3, our model indicated that A β -induced astrocytic calcium hyperactivity depended on TRPA1 calcium channel activation. The calcium oscillation frequency of astrocytes with TRPA1 is significantly faster than that without TRPA1, and the contribution of TRPA1 to the abnormal increase in calcium excitability gradually increases with higher A β concentration.

Amyloid- β -induced Ca^{2+} -dependent glutamate release resulting in neuronal hyperactivity

Our model replicated this finding, demonstrating that as the A β concentration increased, the amplitude and frequency of astrocytic calcium oscillations increased. Consequently, the increased levels of glutamate in the extracellular space not only enhanced the probability of glutamate release from presynaptic neurons but, more importantly, affected the firing frequency of postsynaptic neurons, leading to excitotoxicity.

When the A β concentration is relatively low, i.e., $a = 0.2$, astrocytic Ca^{2+} oscillations have a lower frequency, resulting in reduced glutamate release (Figure 4). Conversely, when $a = 0.8$, astrocytic calcium oscillations double in frequency, causing a significant increase in glutamate release. This elevated release of glutamate results in a marked increase in the postsynaptic neuron firing rate, indicating a state of hyperexcitability. Additionally, the increase in astrocytic Ca^{2+} levels also enhances the probability of glutamate release from presynaptic neurons. Further details of the impact of astrocytes on postsynaptic neuron firing can be observed in Figure 4.

Quantifying the firing frequency of postsynaptic neurons revealed that with increasing " a " values, which corresponded to higher A β concentrations, the firing frequency of postsynaptic neurons showed an upward trend (Figure 5). Each large fluctuation of the trajectory in Figure 4A reflected the regulation of astrocyte calcium excitation on neuronal discharge. In particular, in the dotted frame part, when $a = 0.8$, the black calcium oscillation peak coincides with the peak of the change of the discharge frequency of the postsynaptic neurons in the lower column. The results show that in the early stages of AD, astrocytes, under the inductive effect of A β , are fully capable of regulating the excitability of synaptic neurons by releasing gliotransmitters, contributing to the establishment of A β -induced neuronal hyperexcitability and thereby causing a series of consequences such as synaptic dysfunction and cognitive decline.

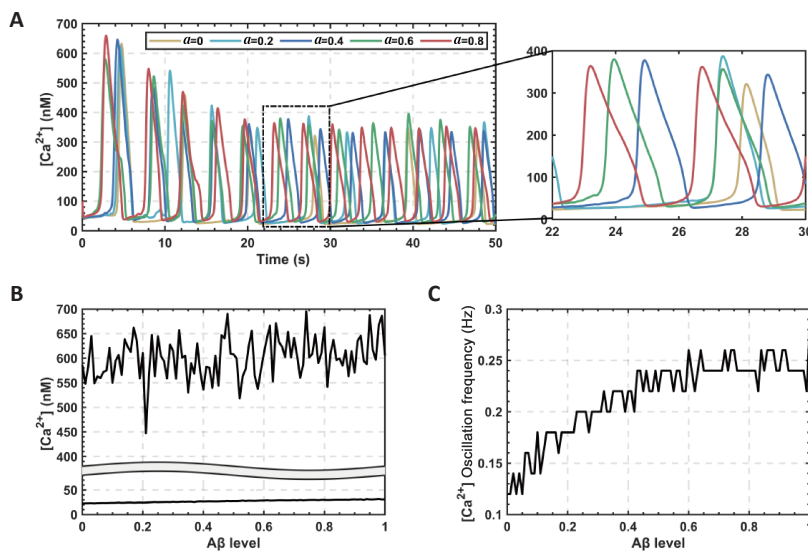


Figure 2 | Effect of A β on astrocytic Ca^{2+} concentrations.

(A) The waveforms of calcium oscillations at different A β levels ($\alpha = 0, 0.2, 0.4, 0.6, 0.8$). (B) The variation in the minimum and maximum amplitudes of astrocytic calcium oscillations at different A β levels. (C) Changes in the frequency of astrocytic calcium oscillations at different A β levels. The threshold for calculating the frequency of calcium oscillations in astrocytes was set to 200 nM. In addition, the calcium oscillation waveform of astrocytes may exhibit a phenomenon where a peak has not yet decreased to the baseline calcium concentration and then begins to rise. For these two consecutive peaks, calcium peaks with a height of less than 200 nM on both sides were not considered for quantification. During the statistics, each round of simulation is conducted 10 times, and the average value is taken as the final statistical data. A β : Amyloid- β .

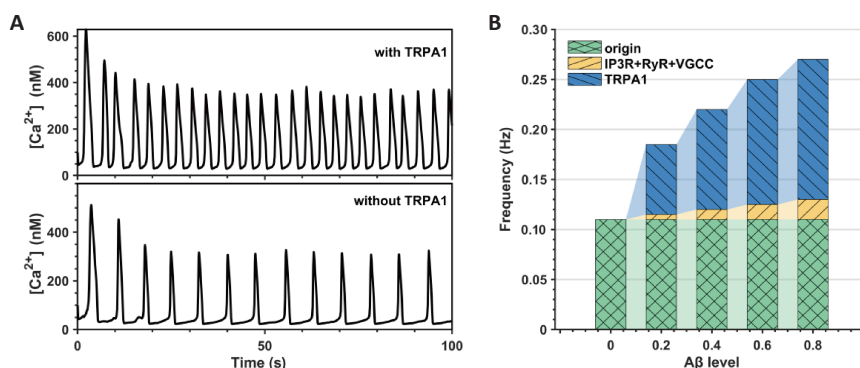


Figure 3 | Contribution of TRPA1 channels to the alteration of calcium excitation in astrocytes.

(A) Waveforms of calcium oscillations with and without the TRPA1 channel. (B) The contribution of A β to astrocytic Ca^{2+} dynamics through different pathways. During the statistics, each round of simulation is conducted 10 times, and the average value is taken as the final statistical data. A β : Amyloid- β ; IP3R: xxx; TRPA1: transient receptor potential ankyrin 1; VGCC: voltage-gated calcium channel.

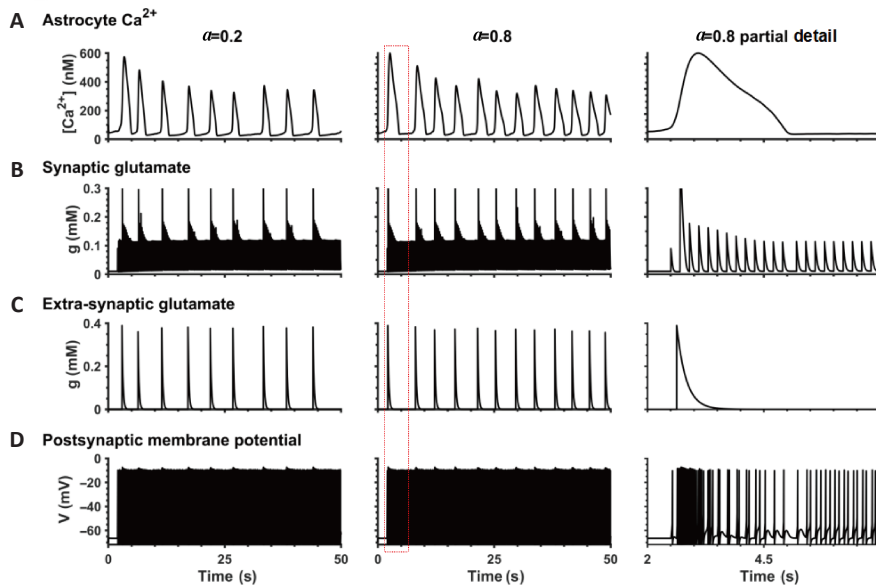


Figure 4 | The impact of Ca²⁺-dependent glutamate release induced by amyloid-β on neuronal excitability. (A–D) Astrocytic calcium oscillations (A), synaptic glutamate (B), extra-synaptic glutamate (C), and postsynaptic neuron membrane potential (D) when $\alpha = 0.2, 0.8$. The right column corresponds to a partially enlarged detail in the middle column, shown as the red dashed box.

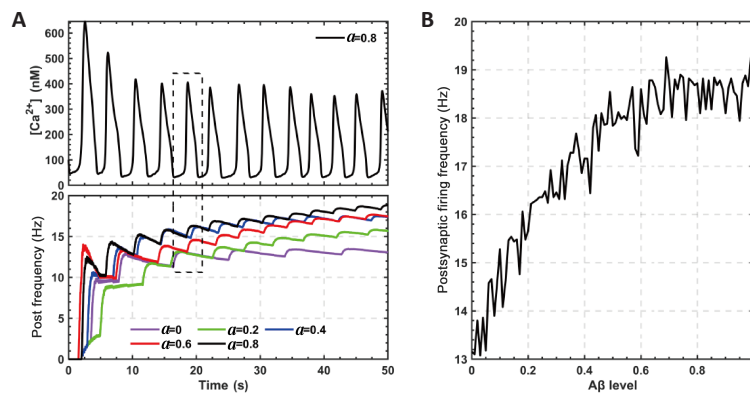


Figure 5 | Effects of astrocyte Ca²⁺-dependent glutamate release on the neuronal firing rate. (A) Changes in the firing rate of postsynaptic neurons during 30 seconds of simulation at different amyloid-β levels ($\alpha = 0, 0.2, 0.4, 0.6, 0.8$). (B) Firing rates of postsynaptic neurons at different amyloid-β levels. The firing rate in A is calculated via a moving average. For the statistical postsynaptic neuron firing frequency, the threshold is set to -20 mV. During the statistics, each round of simulation is conducted 10 times, and the average value is taken as the final statistical data.

Increased gamma-aminobutyric acid release mediated by gamma-aminobutyric acid transporter-3 under excitotoxic conditions

In AD, excitotoxicity is considered to be a multi-phase event that may be related to imbalance of the astrocyte-neuron signaling pathway. We have previously successfully shown that enhanced glutamate release mediated by astrocytes can indeed cause excitotoxicity. Moreover, multiple experiments have revealed increased GABA release from reactive astrocytes near Aβ plaques (Jo et al., 2014; Brawek et al., 2018). These findings indicated that the changes in glutamate and GABA are interrelated and jointly affect the E/I balance of neurons.

Each calcium oscillation in astrocytes increases the probability of glutamate release by presynaptic neurons (Figure 6). The released glutamate is taken up by EAAT-2, and this uptake is accompanied by the influx of Na⁺, leading to an increase in intracellular Na⁺ concentration within astrocytes. This increase causes a reduction in the reverse potential of GAT-3, which, in some cases, may decrease to a value lower than the astrocyte membrane potential. Ultimately, GAT-3 can be triggered to shift its transport direction from uptake to release, resulting in an increase in synaptic GABA concentration (Figure 6C), which stabilizes at a certain level when the system reaches equilibrium. Additionally, the graph illustrates that as the GABA concentration increases, the baseline of the GAT-3 reverse potential gradually rises, making it less likely to exceed the threshold for changing its transport direction. When $\alpha = 0$, astrocytic calcium excitability is relatively low, which has a minor impact on the probability of glutamate release by presynaptic neurons and results in a relatively low synaptic glutamate level, and, ultimately, a lower stable synaptic GABA concentration. However, when $\alpha = 0.8$, the frequency of astrocytic calcium oscillations increases, causing frequent and substantial spikes in synaptic glutamate concentration and consequently elevating the stable synaptic GABA concentration (Figure 6C).

Enhanced synthesis of glutamate and gamma-aminobutyric acid within astrocytes contributes to gamma-aminobutyric acid transporter-3-mediated gamma-aminobutyric acid release

Similar to neurons, astrocytes are capable of producing and releasing GABA. GABA synthesis by astrocytes is mediated by three pathways in different brain regions, including monoamine oxidase B, glutamate decarboxylase (GAD), and diamine oxidase

(Kilb and Kirischuk, 2022; Liu et al., 2022). The amount of GABA synthesized depends on physiological or pathological conditions. Notably, glutamate can be converted to GABA through GAD, implying that intracellular glutamate and GABA are related to each other to a certain extent while affecting the changes in the EAAT-2 and GAT-3 coupling system. Moreover, research has shown that GABAergic astrocytes are involved in the pathogenesis of various diseases, including AD, stroke, epilepsy, and other neurological disorders (Wu et al., 2014). In the AD mouse model, monoamine oxidase B-mediated GABA synthesis in the hippocampus was enhanced by approximately five times in comparison with the control group, and the memory of AD mice could be restored by blocking it (Jo et al., 2014; Behl et al., 2021). Furthermore, samples of patients with AD showed abnormally high GABA release from astrocytes (Le Meur et al., 2012), along with increased levels of GAD expression (Wu et al., 2014). On the other hand, maintaining low glutamate concentrations within astrocytes is essential for reliable synaptic information transmission (Flanagan et al., 2018), and aberrant glutamate concentrations are likely to result in elevated GABA levels through a chain reaction (Wu et al., 2014). Therefore, the synthesis of glutamate and GABA within astrocytes appears to be a crucial target in AD. Thus, examining how the expression of glutamate and GABA-synthesizing enzymes within astrocytes affects GABA release is essential.

Figure 7 illustrates that increasing intracellular GABA concentrations can promote GAT-3 release, and when the intracellular GABA levels reach a certain threshold, GAT-3 release reaches a critical state. Subsequent increments in intracellular GABA concentrations do not lead to increased GAT-3 release and can only cause a slight difference in GAT-mediated synaptic GABA levels, which may be due to the fact that GAT-3 is predominantly controlled by the sodium gradient. Additionally, the fluctuations in astrocytic neurotransmitter release are reduced, and the time to reach equilibrium is shortened, indicating that the system becomes less susceptible to external glutamate concentrations. These findings may suggest that the GAT-3 release pathway is not significantly influenced by a substantial increase in intracellular glutamate and GABA concentrations, preventing a disproportionate release of GABA. Furthermore, if only one neurotransmitter's concentration is altered, in comparison with glutamate, even small changes in the intracellular GABA concentration are more likely to enhance the release of GABA (Figure 7B), indicating that increased GABA synthesis in AD indeed contributes to the enhancement of astrocyte inhibition.

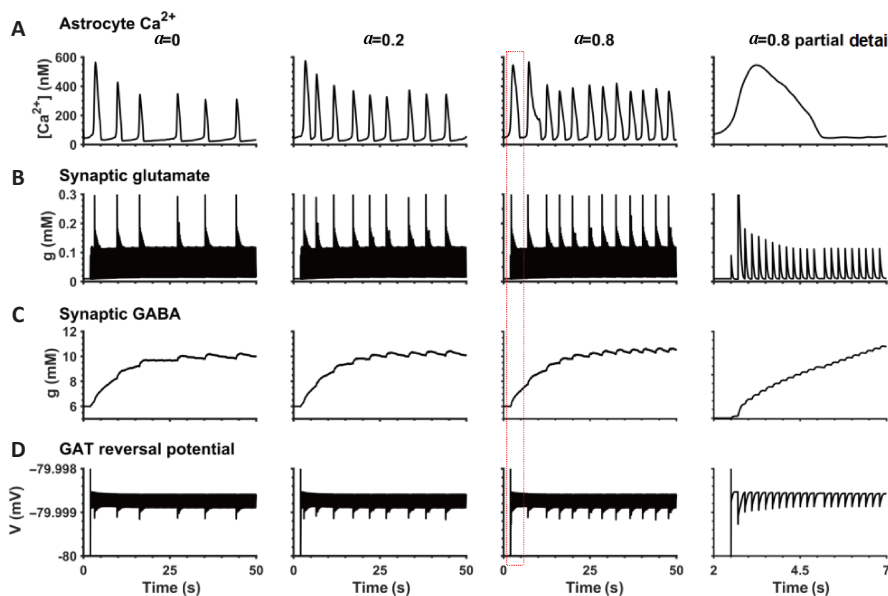


Figure 6 | The impact of Ca^{2+} -dependent glutamate release on GABA transporter-mediated GABA transport.

(A–D) Astrocytic calcium oscillations, synaptic cleft glutamate, synaptic cleft GABA, and GAT reversal potential when $\alpha = 0, 0.2, 0.8$. The right column corresponds to a partially enlarged detail in the middle column, shown as the red dashed box. In these simulations, a presynaptic neuron was subjected to an applied current at 20 seconds with a pulse frequency of 10 Hz. GABA: Gamma-aminobutyric acid; GAT: GABA transporter.

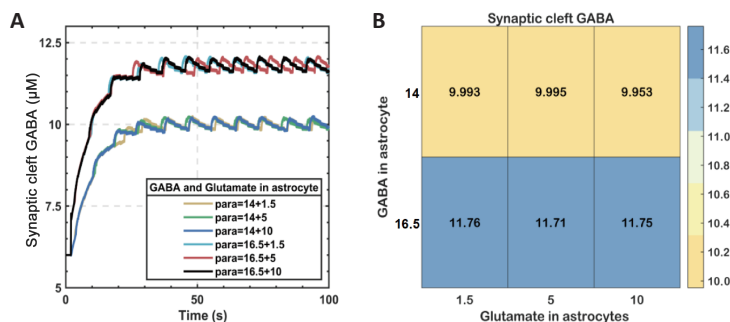


Figure 7 | The impact of intracellular concentrations of glutamate and GABA in astrocytes on GABA transporter-mediated GABA transport.

(A) Changes in synaptic cleft GABA concentrations corresponding to different intracellular GABA concentrations ($GABA_{ast}=14$ mM, 16.5 mM) and glutamate concentrations ($Glu_{ast}=1.5, 5$, and 10 mM) in astrocytes. (B) The average synaptic cleft GABA concentration corresponding to different intracellular GABA concentrations ($GABA_{ast}=14$ and 16.5 mM) and glutamate concentrations ($Glu_{ast}=1.5, 5$, and 10 mM). The numerical values are obtained from the average synaptic cleft GABA concentration after 50 seconds of simulation. During the statistics, each round of simulation is conducted 10 times, and the average value is taken as the final statistical data. GABA: Gamma-aminobutyric acid.

Amyloid- β reduces neural coding efficiency

For a given stimulus, the total entropy rate is a measure of the repertoire of spiking patterns that can be produced by the compartment, setting its information capacity (Strong et al., 1998). We measured the total entropy rate by presenting different spike trains through varying $A\beta$ concentrations in each trial. Repeated presentations of the same stimulus (with an unchanged $A\beta$ concentration) did not produce identical spike trains, and this variability was quantified as the noise entropy rate (Strong et al., 1998). The total spike train entropy rate and noise entropy rate both increased with elevated $A\beta$ concentrations (Figure 8A); the noise entropy was relatively small due to the fixed 10-Hz stimulus applied to the presynaptic neurons in our model, indicating that it was only affected by astrocytes. The information provided by spike trains is the difference between the total spike train entropy rate and this noise entropy rate, and serves as a direct measure of information content without assumptions about how information is represented and its meaning (Strong et al., 1998). The information rate also increases with increasing $A\beta$ concentration (Figure 8B). By dividing the information rate by the corresponding spiking rate at each $A\beta$ concentration, the information encoded by each spike, i.e., encoding efficiency, can be determined. The encoding efficiency decreased with an increase in $A\beta$ content. Thus, when $A\beta$ begins to have a toxic effect on astrocytes, the nearby neurons are overexcited, and in addition to the reduced encoding efficiency, the frequent action potentials also greatly increase the energy consumption, which may lead to synaptic disorders and neurodegeneration and thereby affect the normal learning and memory function of the brain.

Discussion

In this study, by integrating the bidirectional mechanisms of interaction between astrocytes and neurons relevant to AD, we presented a new tripartite synapse model that simulates the effects of $A\beta$ accumulation on astrocytic homeostasis and subsequent neuronal excitability and coding efficiency. This proposed model has combined 5 published modeling works on tripartite synapse (Tsodyks et al., 1998; Tewari and Majumdar, 2012; De Pitta and Brunel, 2016; Flanagan et al., 2021; Liu et al., 2023), which is a more comprehensive model with key advancements include: integration of TRPA1-mediated $A\beta$ effects, dynamic coupling of EAAT-2/GAT-3 with intracellular ion gradients. These unique features make this model different from our previous model with the morphological modeling (Liu et al., 2023). Also, we have applied information entropy to

quantify the firing encoding. Our putative blueprint for the development of AD will help to find effective targets for AD and generate the final comprehensive disease theory. Our findings suggest that astrocytes play a pivotal role in the E/I balance of brain function and represent a crucial target for preventing or even reversing the progression of AD.

In our work, we effectively replicated experimental findings showing that $A\beta$ induces excessive calcium activity in astrocytes through TRPA1. We changed the agonist concentration of the TRPA1 allosteric model to reflect the excitatory effect of $A\beta$, and assumed that the $A\beta$ concentration represents the severity of AD onset. Our data indicated that with an increase in $A\beta$ concentration, baseline calcium oscillations showed a slight increase, with a notable increase in frequency. Moreover, TRPA1 can control the release of gliotransmitters to influence the excitability of synaptic neurons. Therefore, we propose that the release of glutamate from astrocytes into the extracellular space would not only affect the release probability of presynaptic neurons but also directly affect the firing frequency of postsynaptic neurons. Our model showed that as the $A\beta$ concentration increased, postsynaptic neurons became excessively excited, indicating that changes in astrocyte excitability in the early stages of AD play a significant role in the subsequent neuronal hyperexcitability and even synaptic dysfunction. Third, maintaining normal extracellular glutamate concentration is crucial for synaptic function, and astrocytes take up extracellular glutamate through EAAT-2 to ensure that synaptic glutamate levels remain within a certain range to maintain neuronal excitability at an optimally moderate level. Additionally, astrocytes can also modulate the E/I balance by altering the extracellular GABA concentration through GAT-3. EAAT-2 and GAT-3 are interlinked and are influenced by gradients of Na^+ concentration across the membrane, allowing them to interdependently regulate extracellular glutamate and GABA concentrations. Here, we considered how astrocytes maintain or disrupt the E/I balance when extracellular glutamate concentration is elevated, causing excitotoxicity, under the attack of $A\beta$ in AD. Simulation results showed that an increase in extracellular glutamate concentration promoted GABA release by GAT-3 through the modulation of EAAT-2 reversal potential and the intracellular Na^+ concentration. This outcome may reflect the protective effect of astrocytes in the brain. When the synaptic glutamate concentration is sufficiently high to excessively activate neurons, the E/I balance is destroyed. Astrocytes can sensitively detect such changes, and they subsequently enhance the inhibitory effects through the coupling of EAAT-2 and GAT-3 in the case of excitotoxicity, facilitating restoration of E/I balance.

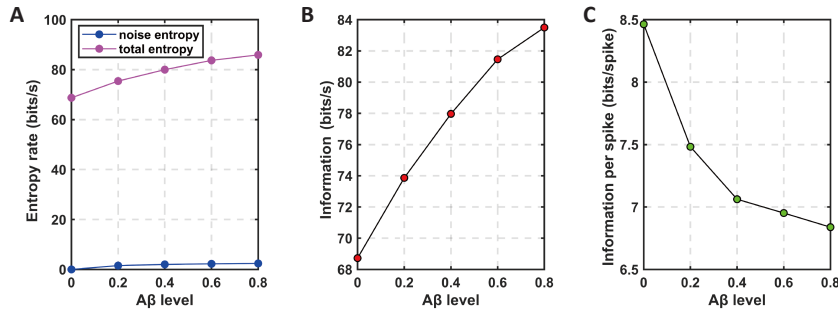


Figure 8 | Entropy, information rate and information per spike under different Aβ concentrations.

(A) The total entropy and noise entropy of the spike trains generated by the Aβ concentration ($\alpha = 0, 0.2, 0.4, 0.6, 0.8$). (B) It calculates the difference between the total entropy and the noise entropy, which is the information rate. (C) It calculates the information per spike. During the statistics, each round of simulation is conducted 10 times, and the average value is taken as the final statistical data. Aβ: amyloid-β.

We also investigated the influence of intracellular glutamate and GABA synthesis on GAT-3 release. We found that increasing the levels of both can lead to an increase in the release of GABA by GAT-3. Thus, alongside the increase in extracellular glutamate concentration, enhanced intracellular synthesis in patients with AD can further augment astrocytes' strong inhibition of nearby neurons, thereby promoting restoration of the E/I balance. Experimental evidence has shown that the GABA content in astrocytes increases during the early stages of Aβ deposition and reaches its peak when Aβ plaque deposition begins, which is followed by a decrease. This increase is likely not directly influenced by Aβ levels but may be caused by excessive activity in nearby neurons (Brawek et al., 2018). On mutual corroboration with these experimental findings, our model also demonstrated that astrocytes can convert neuronal hyperexcitability into strong inhibition of neighboring neurons through direct coupling between glutamate uptake and GABA release, which may reflect a compensatory inhibitory mechanism to restore the disrupted E/I balance in the brain affected by Aβ plaque deposition. On the basis of our previous modeling results, we speculated that before the deposition of Aβ plaques, reactive astrocytes have over-activated nearby neurons through Ca^{2+} -dependent gliotransmitters, aggravating excitotoxicity. Subsequently, this triggers the compensatory mechanisms of astrocytes, such as the reversal of GAT-3-mediated transport direction and increased GABA synthesis to promote GABA release. This enhancement of GABA inhibition helps maintain the E/I balance in the brain.

EAT-2 and GAT-3 in the model are extremely sensitive to the gradient of Na^+ concentration across the membrane, and near-membrane $[Na^+]_i$ transients determine the direction of GABA transport by GAT-3, indicating that small changes in Na^+ concentrations are directly correlated with the ratio of extracellular GABA and glutamate concentrations. Thus, in addition to astrocyte calcium homeostasis, the local E/I balance is also affected by astrocyte ion homeostasis, including Na^+ homeostasis. In particular, the emerging data from Ca^{2+} and Na^+ imaging combined with imaging of extracellular glutamate and GABA highlight the need for a biophysical perspective to unify these findings (Brazhe et al., 2023)—neurotransmitters and Ca^{2+} transporters are driven by the Na^+ gradient and are sensitive to transmembrane potential modulation by $[K^+]_o$, which, in turn, regulate neuronal excitability and presynaptic release probability.

Adjustable, flexible, and balanced synchronization between brain regions is the primary mechanism that supports fast and efficient information processing, and the balance between excitatory glutamatergic and inhibitory GABAergic activity is essential for achieving synchronization. Our modeling simulated the fact that the influence of Aβ is marked by excessive firing of synaptic neurons and a consequent decrease in encoding efficiency. The reduction of information transmission efficiency affects the stability of information flow through circuitry, causing cognitive impairment and memory loss. The excessive excitability caused by Aβ results in increased metabolism, accumulation of garbage, and successive inflammation (Gordleeva et al., 2020). This cascade accelerates neurodegeneration and AD progression.

Our model suggests that the decline in cognitive abilities in AD is likely the result of multiple interacting mechanisms. Therefore, a combination of therapeutic approaches targeting various pathological features should be employed in the early stages of AD (Behl et al., 2021). Targeting astrocytes in the early stages of AD for therapeutic interventions may be the most effective approach. However, the biphasic role of reactive astrocytes under pathological conditions, which depends on the beneficial or harmful effects of various factors, requires attention. The levels of tau alterations and variations in the density of Aβ plaques across areas show clear differences, indicating that AD attacks brain regions in a distinct order. Coupled with the stage changes in AD development, this spatiotemporal heterogeneity highlights the need for greater flexibility in formulating treatment strategies. In particular, the results of our model confirm that astrocytes can initially mediate excitotoxicity in neurons, but can subsequently also automatically generate compensatory responses to exert protective effects. Thus, the status of astrocytes at different points in time and space should be rationally evaluated, and therapeutic approaches that can enhance or inhibit their effects should be chosen to facilitate better control of astrocytes. Specifically, on the one hand, attempts can be made to restore calcium homeostasis to prevent subsequent overexcitation of astrocytes and, for example, deactivation of TRPA1 channels. On the other hand, enhancing the inhibitory function of astrocytes can normalize nearby overexcited neurons. Thus, by modulating the glutamine-glutamate/GABA cycle, moderately enhanced GABA synthesis in astrocytes can increase synaptic GABA concentration.

Our model had some limitations. In addition to Aβ, tau protein has been suggested to be significantly involved in AD (Ye et al., 2024). For simplicity, the current model did not account for the mechanisms of tau protein. Future studies should address this limitation by accounting for more possible pathways. For instance, future iterations of the model could include the Best-1 channel. GAT-3-mediated GABA release shows a negative feedback mechanism. Our findings suggested that the strength of GAT-3-mediated GABA release is insufficient to alter excitotoxicity and may only fine-tune it under pathological conditions. The existing literature includes conflicting experimental results suggesting that astrocytes release GABA in AD through GAT3 or Best-1 (Kilb and Kirischuk, 2022). Due to the bell-shaped relationship between the increase in GABA levels in reactive astrocytes and the accumulation of Aβ plaques (Brawek et al., 2018), the GABA level in astrocytes and the mechanism of active GABA release may depend on the stage of the disease. Local ion signaling and GABA gradients within astrocytes may play decisive roles in the activation/closure of specific GABA-release mechanisms. Elucidating the mechanisms controlling GABA release could facilitate the development of therapeutic tools for pathological conditions. Finally, although this was not mentioned in our model, GAT-3 can also induce astrocytes to release ATP through NCX, which acts on presynaptic neurons to reduce glutamate release and thereby downregulate excitatory transmission. Overall, our study presents a relatively systematic theoretical modeling work on AD considering the tripartite synaptic excitation/inhibition balance mechanism, but the findings still need further improvement and experimental verification.

Author contributions: Conceptualization, writing – review & editing: JL, SC, JKL, AZ, and MW. Data curation: JL and MW. Formal analysis, writing – original draft, investigation: JL and SC. Funding acquisition: SC and AZ. Methodology: JL, SC, JKL, and AZ, MW. Project administration, supervision: SC. Resources: SC, AZ. Software, visualization: JL. All authors approved the final manuscript.

Conflicts of interest: The authors declare that they have no conflicts of interest.

Data availability statement: All relevant data are within the paper and its Additional files.

Code availability statement: Simulation code was written in Matlab R2022a. The code is available in the author's GitHub page (<https://github.com/xxx/tripartite-synapse-model>).

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Additional file:

Additional Table 1: Variables and parameters used in the proposed model.

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