Kinetic model of the glutamate neuron-astrocytic system. N-acetylaspartylglutamate and glutamate carboxypeptidase

Sergey D. Varfolomeev[1,2,3], *Valeriy I. Bykov*[3], *Svetlana B. Tsybenova*[3,*]

[1]Institute of Physicochemical Foundations of the Functioning of Neural Network and Artificial Intelligence, Lomonosov Moscow State University, 119991, Moscow, Russia

[2]Department of Chemistry, Lomonosov Moscow State University, 119991, Moscow, Russia

[3]Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 119334, Moscow, Russia

+79153049387, s.tsybenova@gmail.com

ABSTRACT

Kinetic model of excitatory synapse functioning with glutamate and N-acetylaspartylglutamate (NAAG) as neurotransmitters in a three-component system regarding neuroglial astrocytic cells and the glutamate carboxypeptidase (GCPII) enzyme has been developed. The biphasic nature of the process providing excitation amplification within the modeling framework is shown. The role of excitatory synapse intensity has been investigated. The role of NAAG for enhancing neuronal connectivity and the realization of cognitive function is shown. The mechanism of cognition control by influencing on the efficiency of mGluR3 metabotropic receptor functioning is discussed. The role of GCPII inhibition as a method of improving cognitive functions is studied. Dynamic features of the glutamatergic system behavior in neurodegenerative diseases (Alzheimer's disease, dementia), schizophrenia, traumatic brain injury, and epilepsy, as a part of devel-

opment of the kinetic model are analyzed. The positive role of GCPII inhibition with the NAAG level increase as an effective approach to neuropathology treatment is shown.

Keywords: kinetic model, glutamate, N-acetylaspartylglutamate, glutamate carboxypeptidase, glutamatergic synapse, biphasic nature, cognitive function, neuropathology

INTRODUCTION

The mechanism of the human neurosystem functioning as a molecular biocomputer is the operational interaction of a great number of ion-molecular systems, with the dominant role of interneuron synaptic transmission systems for excitation using specialized synaptic structures. Synapses are 'chemical semiconductors' that implement directed one-way signal transmission (excitation transfer) from one neuron to another. Kinetic modeling of the synapse functioning is an effective method to analyze the mechanism of synaptic transmission and the basis for creating methods of neurosystem control. A kinetic model of cholinergic synapse which is one of the basic elements of the brain neuronal system functioning has been developed [1–3]. Acetylcholinesterase - the enzyme that hydrolyzes acetylcholine, the neurotransmitter with choline and acetic acid formation - acts as the fundamental element in the mechanism of cholinergic synapse 'operation'. The enzyme interlocking by proton, resulting the acetic acid formation, is a hypothesis which explains the information recording and storage mechanism (molecular synaptic memory mechanism) in the neuronal system. It has been established that cholinergic synapses are some of the key elements in the human memory system [4].

The system of glutamatergic synapses is of equal importance for the human brain and is the most representative synaptic system in it. Nearly 40 % of synapses in the human brain are excitatory glutamatergic synapses that transmit excitation by injecting vesicles containing glutamic acid into the synaptic cleft [3]. Glutamatergic synapses are the main excitatory synapses and play a critical role in the nervous system. Being the neurotransmitter, glutamate is the extremely im-

portant component in the memory mechanism [5–19] and is of great importance for the neurodegenerative brain lesion development [5–11]. There is every reason to believe that acute neuropathologies such as stroke, schizophrenia, traumatic brain injury, as well as chronic neurodegenerative diseases such as Alzheimer's disease and Huntington's disease, are associated with excessively high extracellular concentrations of glutamate as an excitatory neurotransmitter in the brain.

The mechanism of glutamatergic system functioning is associated with the participation of N-acetylaspartylglutamate (NAAG), the most represented dipeptide in the brain [20–22]. In the human brain, NAAG concentrations in the range of 10 – 50 mM are detected. This neuropeptide is of the highest concentration and is undoubtedly the most important neuropeptide in the human brain. It is obvious that elucidation of this component role in the physiology of the neuronal system shall be of special attention. There is a reasonable notion of a significant role of N-acetylaspartylglutamate hydrolase enzyme - a peptidase (GCPII) that 'splits off' glutamic acid from NAAG to form free glutamate and N-acetylaspartic acid (NAA) - in the mechanism of glutamatergic system functioning. The NAA role and the mechanism of NAA hydrolysis with acetic acid and asparagic acid formation are discussed in detail in [23–28]. The molecular polymorphism of NAA-hydrolase is the basis for Canavan disease, a fairly common neuropathology.

The key issue of the brain neurosystem functioning is the problem of the primary sensory signal amplification. The primary sensory signal (visual, audible, temperature, or cognitive) is produced by narrowly limited number of sensory receptors and neurons. This signal eventually causes a powerful molecular-physiological response providing excitation and initiation of a great physiological zone functioning in the central nervous system. This is evident, in particular, in the neuronal system response to visual stimulation. Molecular changes in the visual rhodopsin when visual cortex of the brain is excited cause inclusion of a large neuronal-astrocyte system signal (the excitation zone diameter up to 3 cm) in the response mechanism followed by the signal amplification that leads to microvascular dilatation of the excitation zone (the BOLD effect) [29–

35]. In this regard, neurovascular coupling is an integral and fundamentally important component of the brain functioning. Violation of this conjugation is the basis for numerous neuropathologies.

One of the most realistic hypotheses explaining the possibility of signal amplification prior to the molecular-physiological response is based on the enzyme action. Enzyme inclusion is capable of 10^2 to 10^6 times enhancing the primary physical effect on the system due to its catalytic activity. This was first demonstrated on a model system in [36]. Enzyme activation by unblocking the active center is capable of 10^2-fold per second signal amplification due to enzymatic reaction product accumulation.

It seems highly expedient to develop a kinetic model for the glutamatergic system with NAAG participation and description of possible mechanisms of excitation to be amplified. The model is based on a collection of literature data on the interaction of glutamatergic synapses and the astrocytic system.

RESULTS AND DISCUSSION

Structural and biochemical model of the glutamate neuron-astrocytic system. Actually, a rich experimental material is present that makes functioning of glutamatergic synapses understandable. Demonstration of the interaction of glutamatergic neuronal synapses with astrocytes is the important research result [37–42] (Figure 1).

The glutamate-astrocytic system functioning is provided by the interaction between signal-conducting ionic medium of the neuron, presynaptic and postsynaptic membranes of the glutamatergic synapse, AMPA and NMDA - 'classical' receptor structures of postsynaptic membrane, mGluR1/5 and mGluR3 metabotropic receptors [43–49], and the key enzyme GCPII (glutamate carboxypeptidase) localized on the astrocyte membrane [5, 50–52].

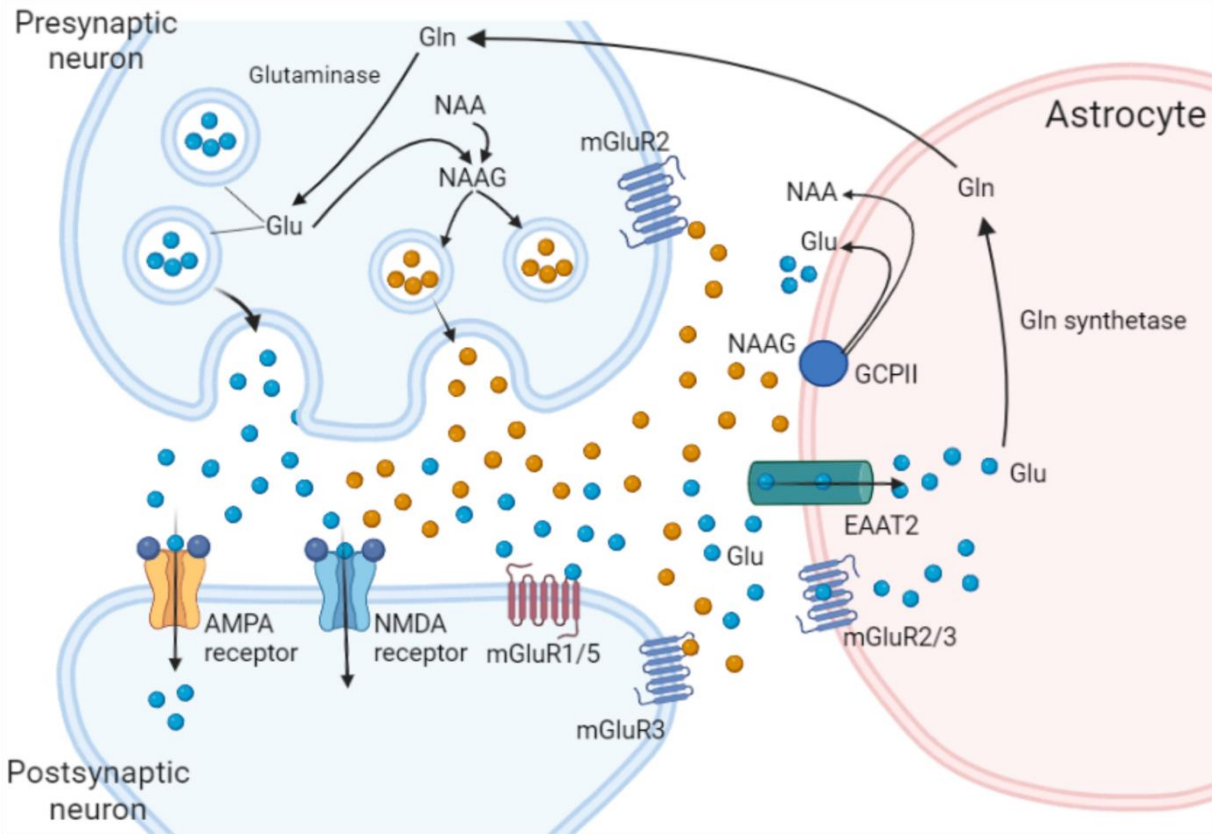

Figure 1. The diagram of glutamate-astrocytic system functioning

It is generally accepted that glutamate and NAAG are released into the synaptic cleft as a result of electromechanical transport initiated by neuronal excitation and fusion of synaptic vesicles with the presynaptic membrane in the synaptic active zone. The glutamate concentration in the synaptic cleft is 0.6 μM at rest and exceeds 10mM when excitation is transmitted during presynaptic depolarization of neurons. Excessive neurotransmitter is effectively removed from the synaptic cleft and transferred to the action zone of the astrocytic system.

The key point of excitative glutamatergic synapses is simultaneous injection of peptide neurotransmitter NAAG and glutamate into the synaptic cleft. As part of the excitation transmission, the NAAG function is to activate the mGluR3 metabotropic receptor located on the postsynaptic membrane. Glutamate carboxypeptidase enzyme (GCPII), which hydrolyzes NAAG forming NAA and free glutamic acid, plays the key role in the response dynamics. The activity level of this enzyme determines glutamate concentration in the system. It is significant that GCPII is the target for drugs under development.

Kinetic model and minimal system of equations. Variable concentrations during the glutamatergic-astrocytic system functioning are Glu (glutamic acid concentration), NAAG (neurotransmitter NAAG concentration), NAA (N-acetylaspartic acid concentration), D (metabotropic receptor functioning products concentration), and N (the number of excited [signal conducting] neurons).

The minimum system of equations includes:

$$\frac{dGlu}{dt} = \left(a_1(Glu_0) \cdot e^{-\alpha_1 t} - \beta \cdot Glu + \frac{V_m NAAG}{K_m + NAAG} + \chi_1 \cdot D \right) \cdot N. \quad (1)$$

The first term of Equation (1) describes the Glu injection dynamics into the synaptic cleft (when modeling the kinetic features of cholinergic system functioning, a similar approach was used [1–3]). The second term of the Equation describes Glu dissipation and absorption dynamics in the synaptic cleft, including the neurotransmitter transfer into the astrocyte action system. The third and the fourth terms of the Equation reflect enzymatic accumulation of the neurotransmitter by means of the GCPII hydrolytic system and the functioning of metabotropic receptors. The factor N in Equation (1) reflects dynamic participation of a group of neurons during excitation.

$$\frac{dNAAG}{dt} = a_2(NAAG_0) \cdot e^{-\alpha_2 t} - \delta NAAG - \frac{V_m NAAG}{K_m + NAAG} + \omega \cdot Glu. \quad (2)$$

The terms in Equation (2) have a similar meaning.

The dynamics of changes in the concentration of metabotropic receptor activation products is described by Equation (3)

$$\frac{dD}{dt} = \xi \frac{R_2 NAAG}{K_2 + NAAG} - \lambda D. \quad (3)$$

Proportionality of the number of neurons located in the excitation zone to the excitative neurotransmitter concentration shall be taken into consideration accordingly:

$$N = \varphi Glu + \chi_2 NAAG, \quad (4)$$

$$\frac{dNAA}{dt} = v + \frac{V_m NAAG}{K_m + NAAG} - \frac{V_{ma}(NAA)^3}{K_{ma} + (NAA)^4} - \rho \cdot NAA. \quad (5)$$

The kinetic model of the glutamatergic synapse interaction with the astrocyte system (1)–(5) describes the process dynamics in two modes: fast (in milliseconds) and slow (in seconds). Variation of the model parameters (a_1, Glu_0, a_2, $NAAG_0$, V_m, ξ etc.) helps in achieving the most significant parameters that may significantly affect the process nature.

The glutamatergic system is the key system for many neurophysiological phenomena. Biphasic nature of response, the excitation amplification system. The fundamentally important components of the synaptic neurosystem response to an external excitative signal are: Glu neurotransmitter; NAAG neurotransmitter injected into the synaptic cleft simultaneously with Glu; AMPA and NMDA postsynaptic membrane receptors; mGluR1/5 and mGluR2/3 metabotropic receptors; the fundamentally important GCPII enzyme localized on the astrocyte membrane and hydrolyzing NAAG with Glu neurotransmitter and NAA formation. A significant number of neurophysiological effects is determined by the dynamic interaction of these system components. The interaction between synaptic transmission of excitation and the enzymatic NAAG transformation process determines the biphasic kinetic response, amplification to impulse excitation response, excitation spreading with the capture of larger area of the neuronal system, and creation of the excitation zone observed experimentally from the BOLD effect.

The phenomenon of signal amplification consists in transition from excitation of a relatively small group of neurons due to a sensory signal to a macroscopic excitation zone creation, which is eventually implemented in the BOLD effect (pulsed dilatation of microcapillaries in the excitation zone initiated by a neurosignal). The experimentally observed kinetic effects of the BOLD signal are described in [31–34]. Figure 2 shows the results of system of Equations (1)–(5) integration. They demonstrate the biphasic nature of the glutamatergic neuronal-astrocytic system functioning. The synaptic 'discharge' occurs in the range of 1ms [54, 55], and the powerful response of the neurosystem has the maximum response in the 1s range.

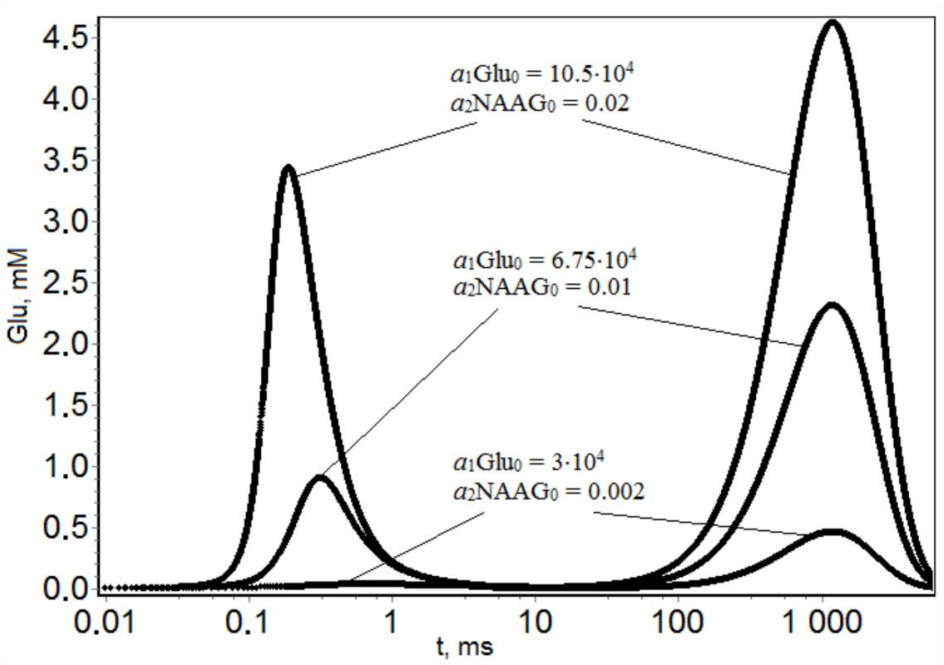

Figure 2. Biphasic nature of the glutamatergic neuronal-astrocytic system functioning (1)–(5) at parameter values: $\alpha_1 = 7 \cdot 10^3$; $\beta = 8 \cdot 10^6 s^{-1}$; $V_m = 4.5 \cdot 10^{-6}$ mM·s^{-1}; $K_m = 130$ nM; $\chi_1 = 100$ s^{-1}; $\alpha_2 = 1.8$; $\delta = 1.5$ s^{-1}; $\omega = 0.2$ s^{-1}; $\xi = 5 \cdot 10^5$; $K_2 = 2$ mM; $R_2 = 0.8$ mM·s^{-1}; $\lambda = 2$ s^{-1}; $\varphi = \chi_2 = 1$ s^{-1}; $v = 0.001$ mM·s^{-1}; $V_{ma} = 0.248$ mM·s^{-1}; $K_{ma} = 1.8 \cdot 10^{-13}$ mM; $\rho = 5$ s^{-1}.

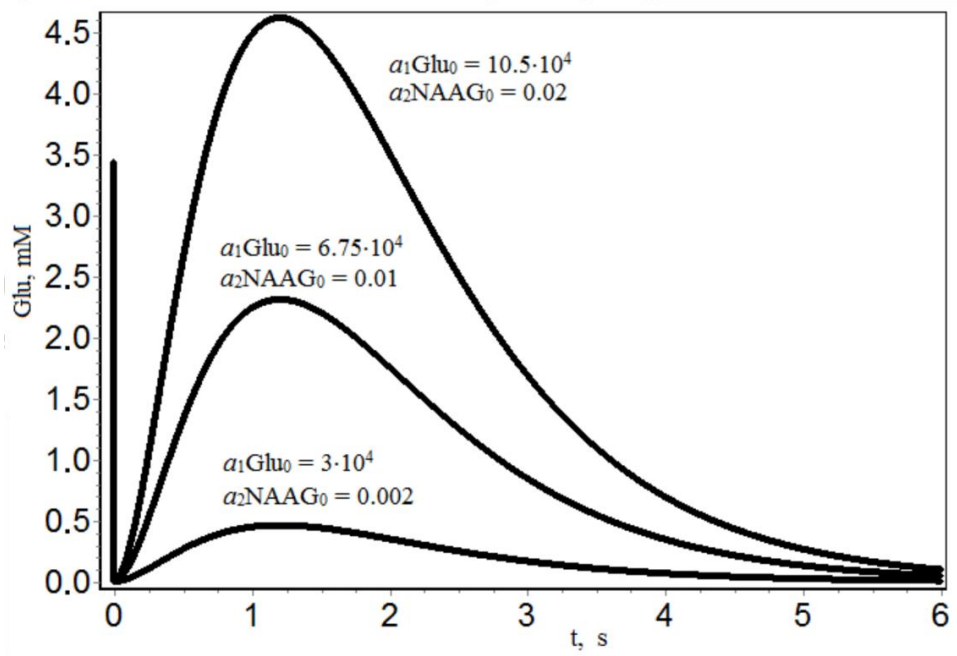

Figure 3. Results of calculating the system of Equations (1)–(5) in the real time scale with varying $a_1(Glu_0)$ and $a_2(NAAG_0)$, which reflect the strength of Glu and NAAG release into the synaptic cleft during exocytosis. The parameter values are the same as in Figure 2.

It should be stressed that Figure 2 shows data in the logarithmic time scale. For the sake of comparison, Figure 3 shows the same data in the real time scale.

Stable and unstable modes of glutamate-astrocyte synapse. Kinetic analysis of the glutamate-astrocyte synapse behavior within the system of Equations (1)–(5) shows that the synapse may demonstrate both stable and unstable (bifurcation) modes. To a considerable extent, this is determined by mediator 'outflow' rates near the reaction zone (β and δ parameters). A decrease in the rate of glutamic acid removal from the reaction zone may lead to an "explosive" excitation of the system (Fig. 4). Figure 4 demonstrates the kinetic behavior with β parameter variation. With high values of β ($\beta < 4 \cdot 10^6$ s^{-1}), the initial impulse 1ms long causes a secondary amplified excitation wave with the time range of 10^3 – 10^5 seconds with the system transition to the stable initial state. NAAG (Fig. 4, *b*) and NAA (Fig. 4, *c*) concentrations, and the number of excited neurons (Fig. 4, *d*) turn back to the initial state. However, a decrease in β from $4 \cdot 10^6$ s^{-1} to $3.95 \cdot 10^6$ s^{-1} at the bifurcation transition point leads to a bifurcation explosion and unlimited growth of Glu, N, and NAAG levels.

NAA concentration reaches the exceptionally high stationary level. The system behaves in a completely similar way when the rate of removal of the second mediator NAAG is varied (Fig. 5).

It is of fundamental interest to the study of the system when the expression level of GCPII (V_m parameter) – the NAAG hydrolysis enzyme – is varied and when this enzyme is inhibited by a potential drug I_0. In the latter case, the equation of the NAAG enzymatic conversion rate is as follows:

$$V_i = \frac{V_m NAAG}{(1 + I_0 / K_i)(K_m + NAAG)}. \quad (6)$$

The analysis shows that the enzyme activity level effect on kinetic responses depends on the zone (stable or close to the bifurcation process), where the enzyme activity variation occurs.

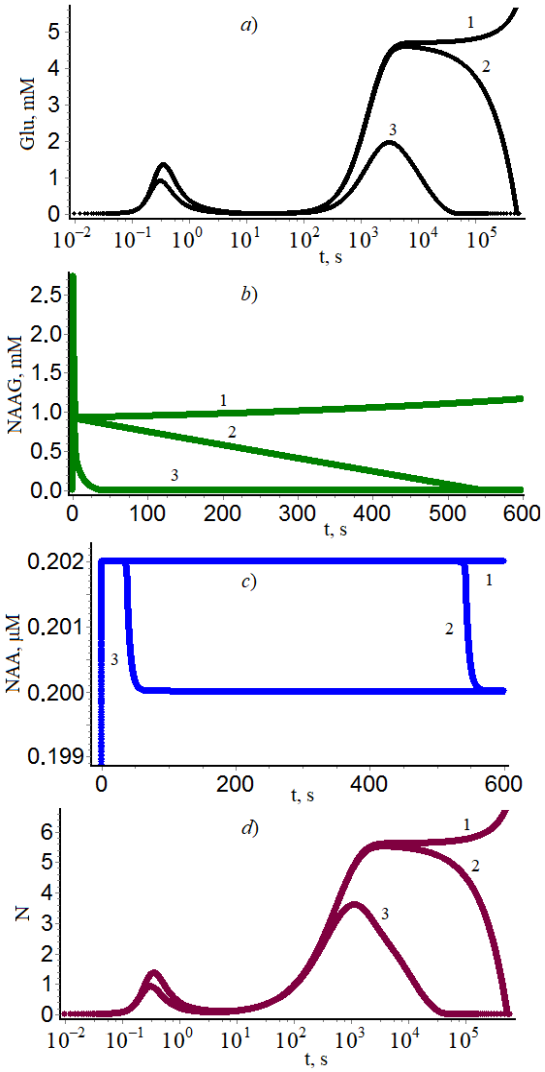

Figure 4. Kinetic responses of the glutamate-astrocyte synapse to β parameter variation: 1 – $\beta = 3.95 \cdot 10^6$; 2 – $\beta = 4 \cdot 10^6$; 3 – $\beta = 8 \cdot 10^6$; (the system of Equations (1)–(5)) *a*) – Glu; *b*) – NAAG; *c*) – NAA; *d*) – N; other parameters are given in the caption to Fig. 2.

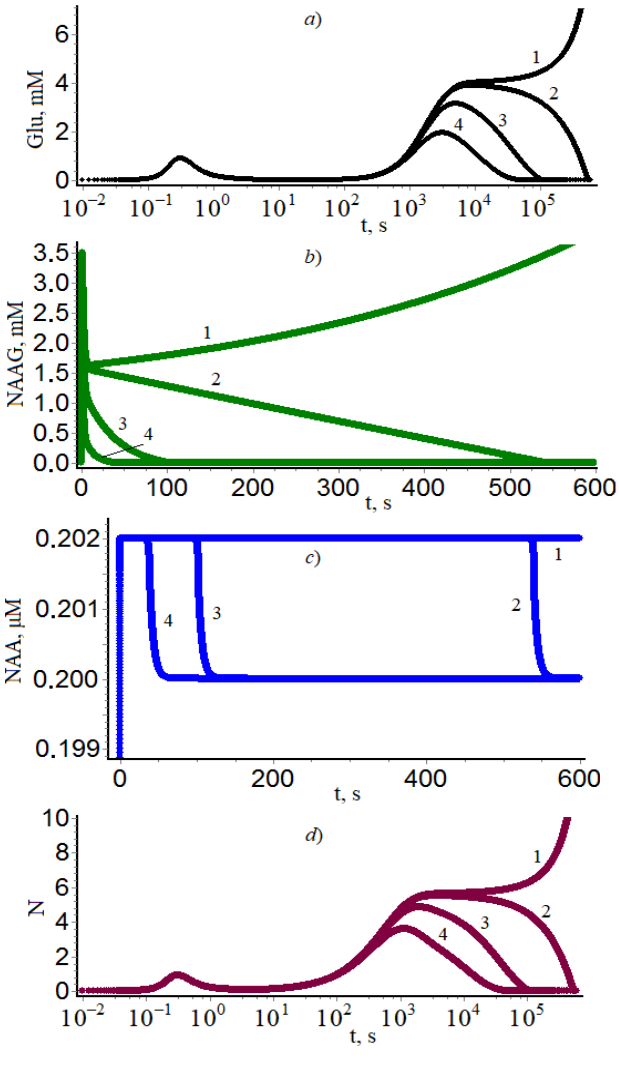

Figure 5. Kinetic responses of the glutamate-astrocyte synapse at variation of δ parameter: 1 – $\delta = 0.49$; 2 – $\delta = 0.5$; 3 – $\delta = 0.6$; 4 – $\delta = 1$; *a*) – Glu; *b*) – NAAG; *c*) – NAA, *d*) – N, other parameters are given in the caption to Fig. 2.

Figure 6 shows the calculation data with V_m variation in the stable zone, $\delta = 1$. It is seen that the system does not virtually react when the key variables change with a sufficiently wide variation of V_m. However, when approaching the unstable zone, the enzyme expression level becomes rather significant (Fig. 7), $\delta = 0.5$.

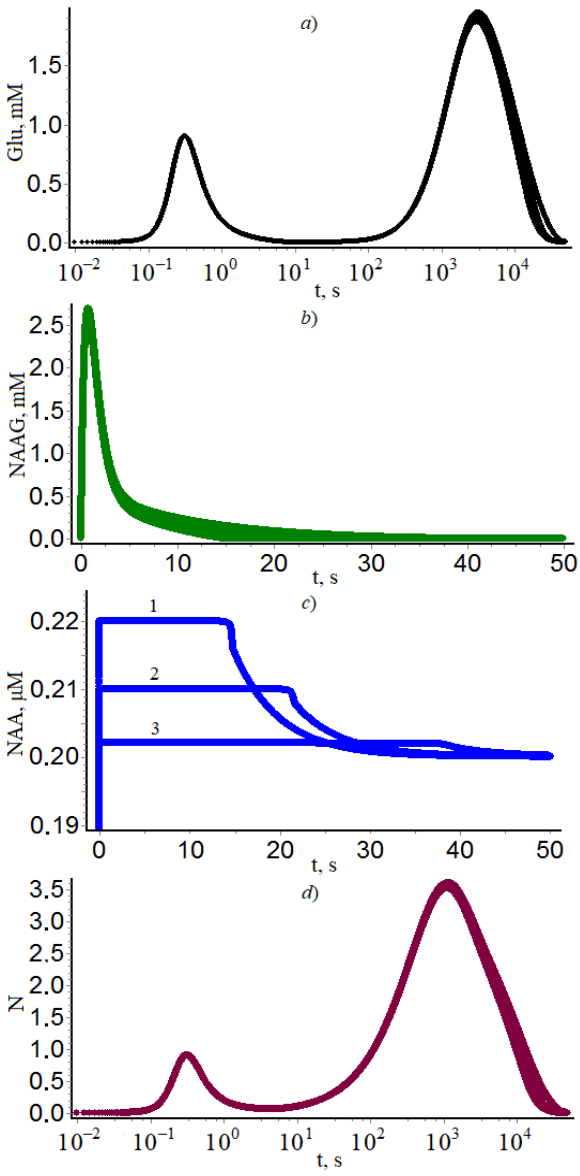

Figure 6. Kinetic responses of the glutamate-astrocyte synapse during GCPII inhibition (V_m variation): 1 – $V_m = 10^{-4}$; 2 – $V_m = 5 \cdot 10^{-5}$; 3 – $V_m = 10^{-5}$; *a*) – Glu; *b*) – NAAG; *c*) – NAA; *d*) – N; in the stable zone ($\delta = 1$).

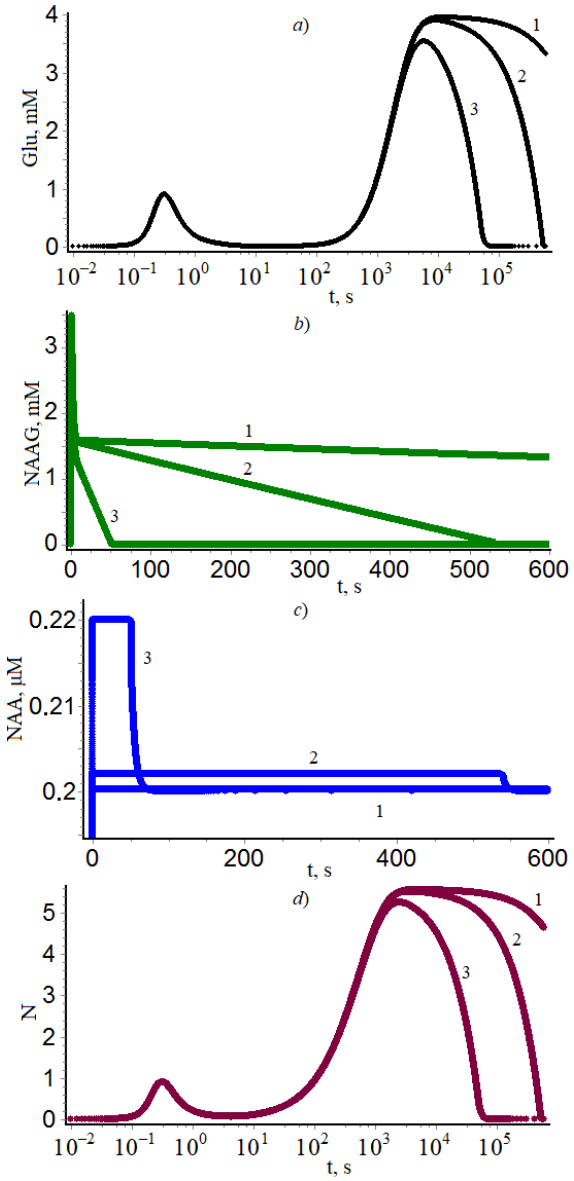

Figure 7. Kinetic responses of the glutamate-astrocyte synapse during GCPII inhibition (V_m variation): 1 – $V_m = 10^{-6}$; 2 – $V_m = 10^{-5}$; 3 – $V_m = 10^{-4}$; *a*) – Glu; *b*) – NAAG; *c*) – NAA; *d*) – N; in the zone approaching the bifurcation process ($\delta = 0.5$).

Similar responses are obtained when the GCPII activity is varied by using inhibitors (Fig. 8). In the zone approaching the bifurcation process, a significant effect of inhibition on the kinetic responses of the system may be observed. It should be noted that strong inhibition of the enzyme ($I_0/K_i = 2.5$; 10) may lead to a high steady-state level of NAAG.

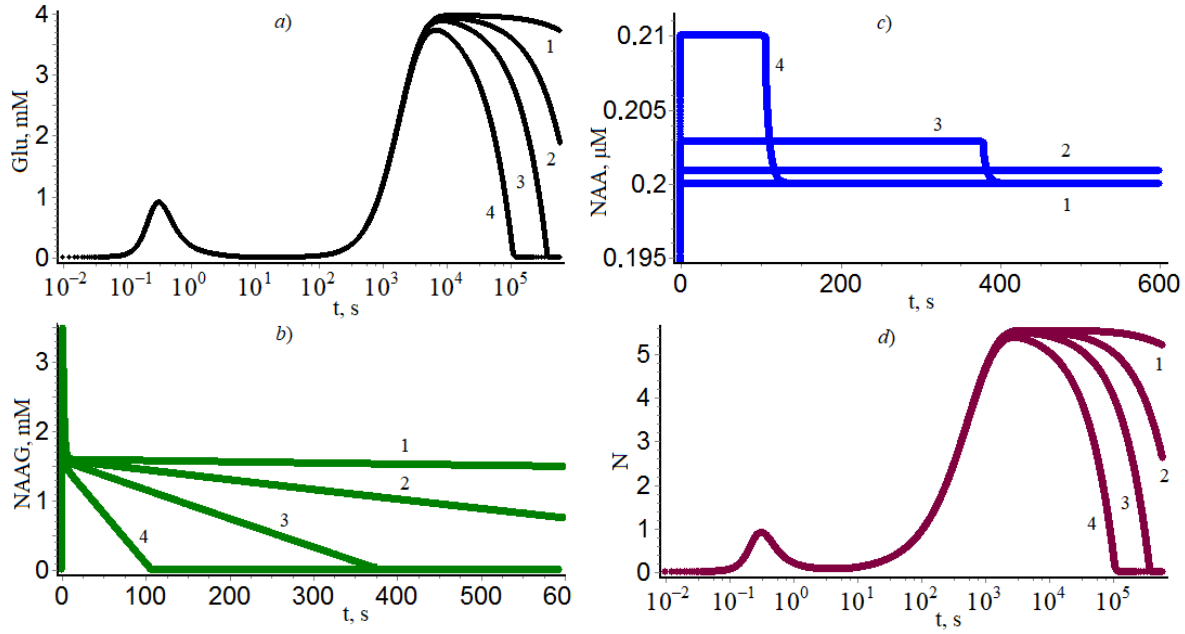

Figure 8. Kinetic responses of the glutamate-astrocyte synapse during GCPII inhibition (I_0/K_i parameter variation: 1 – $I_0/K_i = 10^4$; 2 – $I_0/K_i = 10$; 3 – $I_0/K_i = 2.5$; 4 – $I_0/K_i = 0$; Equation (6) in the zone approaching the bifurcation process, $\delta = 0.5 s^{-1}$.

The fact that the system may transit to the hyperactive excitation state under certain parameters associated with excessive accumulation of Glu and NAAG neurotransmitters (small parameters β and δ) is the important conclusion of the analysis. These kinetic observations may explain the occurrence of epileptic seizures (see the Section *Dynamic behavior of the glutamate synaptic system in neuropathologies* below).

Kinetic data below refer to the analysis of the kinetic system behavior in the stable mode at high β and δ values (modes away from the bifurcation process).

Kinetic behavior of the glutamatergic system during minute-long exposures to light (light excitation, cerebral cortex). Mathematical description of the experimental data. The development of in vivo magnetic resonance spectroscopy technique allowed identification of the main metabolites determining chemical processes in the excitation zone. Increasing the resolving power of the method upon switching to spectroscopy in 7 Tesla fields provide determination of 19 chemical agents in the excitation zone limited by the BOLD effect [56]. It is shown that visual excitation within the BOLD signal boundaries with increasing oxygen concentration reduces

glucose concentration as the main component providing the process energy. As a result, lactic acid concentration increases significantly, by (9 ± 4) %. Excitation is realized by glutamic acid concentration increase, which is the main excitative neurotransmitter, (4 ± 1) %. The light signal elimination returns glutamate concentration to the initial level accordingly. Figure 9 shows the experimental data [56] and mathematical description of the observed effects. The system of Equations (1)–(5) is used as the basis for modeling experimental data with the following modification:

$$\frac{dGlu}{dt} = \left(a_1 Glu_0 \cdot (1 - e^{-\alpha_1 (t - n\Delta t)}) - \beta \cdot Glu + \frac{V_m NAAG}{K_m + NAAG} + \chi_1 \cdot D \right) \cdot N, \quad (7)$$

where n is the number of injection cycles; Δt is the time between impulses (Δt = 6 s).

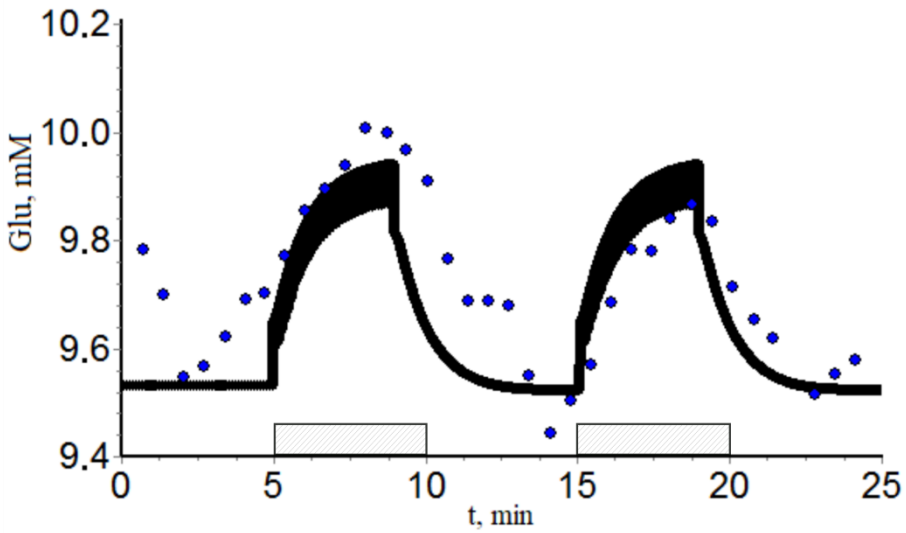

Figure 9. Kinetic changes in glutamic acid concentration of the visual excitation zone within the BOLD effect (cerebral cortex) (experimental data [56] – dots and mathematical description of the process – bold black curve).

The experimental data presented unambiguously illustrate the glutamic acid concentration increase in the excitation zone and allows estimation of the process dynamics.

The role of Glu-excitative synaptic system and NAAG in the cognitive function. Mechanisms of the cognitive control. It is known that mGluR3 receptor stimulation and recognition and learning function (the cognitive function) decreases with NAAG levels [17–19]. On the other hand, inhibition of GCPII glutamate carboxypeptidase increases NAAG concentration, hence,

the physiological response consisting of the working memory improvement. It is also known that increasing expression of the GCPII enzyme gene in humans causes a cognitive decline [19]. Activation of the mGluR3 receptor is associated with accumulation of metabolite D within the model developed. Thus, there are several ways to influence on the system.

1) GCPII inhibition (V_m decrease) –NAAG concentration increase and, as a result, metabolite D concentration increase.
2) Catalytic activity increase for the metabotropic receptor (ξ parameter increase).
3) Metabotropic receptor gene expression increase (V_m parameter increase), which decreases the cognitive function.

The experimental data available for the NAAG level effect on cognition within the model developed allows identification of control points and mechanisms at the excitative glutamatergic system level.

Variation of (cognitive) signal intensity. Impulse discharge of the glutamatergic synapse is determined by $a_1(Glu_0)$ and $a_2(NAAG_0)$ parameters and kinetic parameters α_1 and α_2. First of all, this is determined by the amount of Glu and NAAG neurotransmitters in the presynaptic zone of the synapse, as well as by α_1 and α_2 parameters determining the effectiveness of 'extinguishing' the synaptic discharge. Figure 10 shows dynamics of the pulsed discharge and amplified excitative response (second wave) when $a_1(Glu_0)$ and $a_2(NAAG_0)$ parameters are varied. The 'discharge' and excitation amplification causes a response in NAAG and metabolite D concentrations (Fig. 10), and the number of excited neurons N accordingly.

The efficiency of mGluR3 metabotropic receptor functioning. The quantitative efficiency of metabotropic receptors 'operation' within the model is determined by the parameter ξ. In accordance with Equation (3), the NAAG peptidase concentration is a fundamentally important term. Figure 11 shows the results of integration of the system of Equations (1)–(5) with ξ parameter variation (activation of metabotropic receptors).

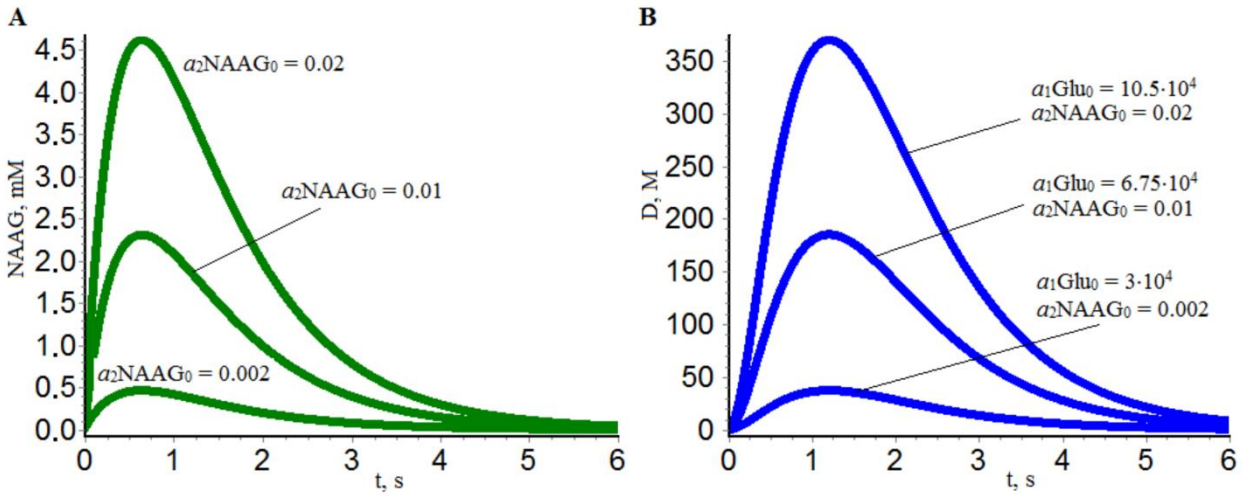

Figure 10. Dynamics of changes in NAAG concentration (**A**) and metabotropic receptors D functioning products concentration (**B**) in the glutamate synapse. The calculation parameter values are the same as in Figure 2.

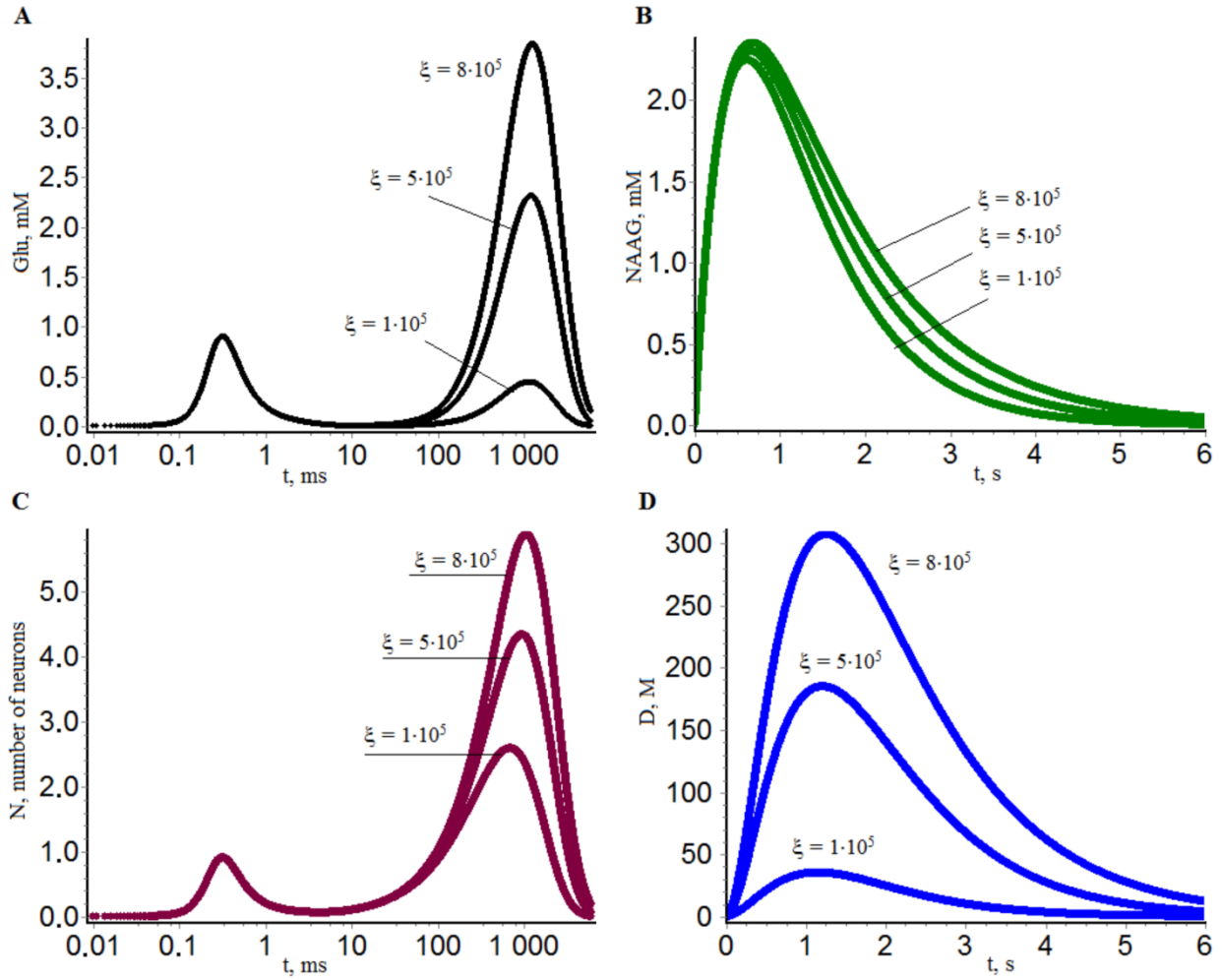

Figure 11. Dynamic response of the system in the concentrations of Glu (A), NAAG (B), D (D) and the number of excited cells N (C) with ξ parameter variation characterizing the efficiency of mGluR3 metabotropic receptor.

Inhibition of glutamate carboxypeptidase GCPII as a method for improving cognitive function.

Exogenous influence on the system behavior may be achieved by inhibiting peptidase, which cleaves glutamic acid from NAAG. Drugs inhibiting this enzyme are currently under active development [12]. GCPII inhibition has been shown to have a positive effect on cognitive functions.

Enzyme inhibition (or its expression level decrease) is reflected by Vm parameter decrease within the model developed. Figure 12 shows the calculated data for Glu, NAAG, D, and N variables with varying V_m. It should be noted that a significant decrease in enzyme activity can lead to interlocking of the synaptic response.

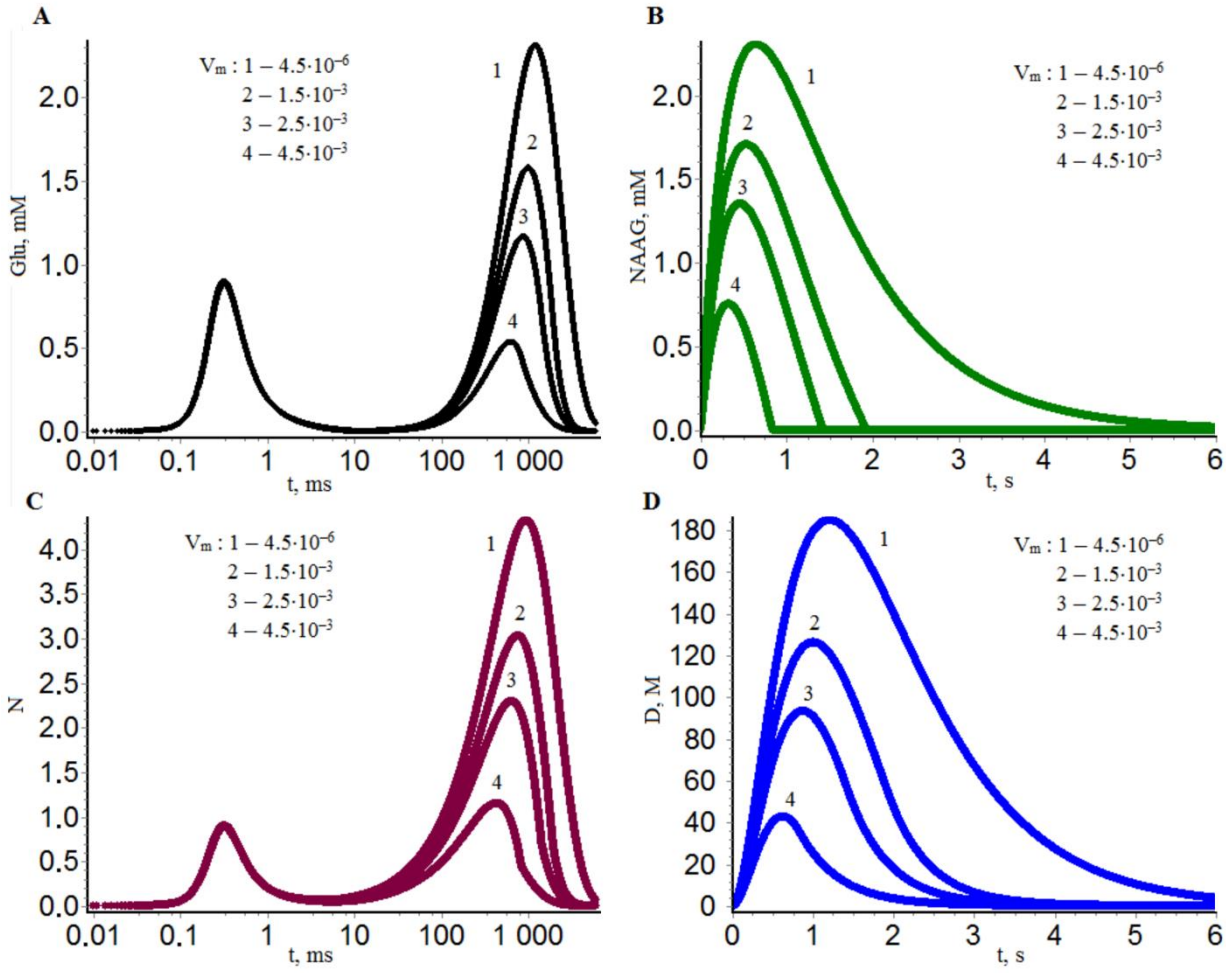

Figure 12. The effect of GCPII activity on dynamic responses of the glutamatergic system. Varying the maximum rate of NAAG hydrolysis (V_m) as affected by GCPII can be implemented by inhibiting the enzyme or its expression level. Calculation parameters are the same as in the caption of Figure 2.

Thus, the cognitive function of the brain, to a certain extent, depends on the functioning of the glutamate-astrocytic system, in which GCPII enzyme plays the important role. In the general case, occurrence of the excitation zone and amplification of the glutamate signal causes excitation transferring to the information recording and storage system which implements the neurological memory mechanism. Within the hypothesis of information recording according to the proton mechanism of cholinergic synaptic system functioning [4], excitation during amplified extension of the excitation zone intersects with the cholinergic system and initiates the memory activation process.

Dynamic behavior of the glutamate synaptic system in neuropathologies. Glutamate synapses, due to the widest representation in the central nervous system, as well as due to the important role in the implementation of cognitive functions (memory, learning), are of importance for the nervous system disorders. Biochemical manifestations of the main components of the system (Glu, NAAG, D, etc.) in the development of neurodegenerative diseases (Alzheimer's disease, dementia, multiple sclerosis), schizophrenia, epilepsy, stroke, and traumatic brain injury are known [12].

Neurodegenerative diseases. For Alzheimer's disease, a significant decrease in the NAAG level in almost all organs of the brain has been shown. This may be caused by the activity (expression level) increase of glutamate carboxypertidase GCPII. The enzyme activity increase causes a decrease in the level of NAAG - the product D of the metabotropic receptor mGluR3 activation, and reduces the number of excited neurons N involved in the response level within the kinetic model considered. These results are shown in Figure 12. On the other hand, the NAAG level increase is achieved by the use of glutamate carboxypeptidase inhibitors. As important exogenous factors of influence, the use of enzyme inhibitors has a positive effect on cognitive functions [5, 12]. This has been shown experimentally.

The fact that GCPII activity decrease causes the glutamate level increase in the system (see Figure 11A) is the unexpected result of the kinetic analysis. This is a consequence of the great role of mGluR3 and product D of its functioning (see Equations (1) and (3)).

Schizophrenia. This neuropathology is a complex disease involving many systems of the central nervous system. The glutamatergic system (the 'glutamate hypothesis') plays a significant role in the disease development [55, 57]. It has been shown that molecular polymorphism of the mGluR3 receptor gene (see Figure 2) is associated with the manifestation of schizophrenia symptoms [58, 59]. Hence, disease manifestations are relieved by receptor ligands [60, 61] and glutamate carboxypeptidase inhibitors [62]. Kinetic modeling during enzyme inhibition predicts NAAG dynamic growth, growth of D, and dynamic increase in the number of neurons involved in excitation in response to an external signal.

Traumatic brain injury. The pathology is comprehensive, including oxidative stress, mitochondrial dysfunction, and multiple inflammatory processes. Obviously, this pathology also affects the glutamate synaptic system dysfunction. The direct neuroprotective effect of GCPII inhibitors in animals with traumatic brain injury was shown in [63–65]. Figure 11 shows kinetic manifestation of such effects.

Epilepsy. Neurophysiological studies demonstrate the critical role of the glutamate synaptic system providing main excitation mechanisms in the development and manifestation of epilepsy [66, 67]. It has been shown that the mGluR3 receptor system activation (within the model developed, the dynamic growth of D component) reduces glutamate amount in the synaptic cleft [68] and relieves a number of pathological manifestations of epilepsy. The GCPII inhibitor affects the system similarly [69]. Figure 12 illustrates possible mechanism for this effect showing NAAG increase with of glutamate carboxypeptidase GCPII activity decrease (inhibition).

These examples illustrate one common important phenomenon: NAAG concentration increase in the system, primarily due to GCPII inhibition, has a positive impact on the general properties of the neurosystem. Namely, it has a positive effect on cognitive functions, has a neuroprotective

and medicinal effect on the development of classical neuropathologies. It's very obvious that GCPII inhibitors have a great pharmacological future.

EXPERIMENTAL AND THEORETICAL METHODS

A kinetic model of excitatory glutamatergic synapse functioning was built on base the results from previous studies [1–4, 12, 27, 56]. The system of ordinary differential equations was integrated using a specially developed program (in the Delphi Community Edition). Visualization of the solutions of the kinetic model was performed by base graphic software.

CONCLUSIONS

In this work, the kinetic modeling of excitatory glutamatergic synapse functioning in a three-component system regarding neuroglial astrocytic cells and the glutamate carboxypeptidase enzyme conducted. The biphasic nature of the glutamatergic neuronal-astrocytic system providing excitation amplification is shown. Kinetic analysis of the glutamate-astrocyte synapse behavior on basis the proposed kinetic model allowed to show that the synapse may demonstrate both stable and unstable (bifurcation) modes.

The results of the model calculation were compared with the experimental data on the changes in the glutamic acid concentration during visual excitation. The mechanism of cognition control by influencing on the efficiency of mGluR3 metabotropic receptor functioning is discussed. GCPII inhibition as a method of improving cognitive functions in the brain is studied. It is shown that GCPII activity decrease causes the glutamate level increase in the system within the proposed kinetic model. Dynamic features of the glutamatergic system behavior in neurodegenerative diseases (Alzheimer's disease, dementia), schizophrenia, traumatic brain injury, and epilepsy, as a part of development of the kinetic model are analyzed. The positive role of GCPII inhibition with the NAAG level increase as an effective approach to neuropathology treatment is shown.

CONFLICT OF INTEREST: None declared

AUTHOR CONTRIBUTIONS

S.D.V. analyzed the experimental and theoretical data. S.D.V. and V.I.B. constructed the kinetic model. S.D.V. wrote the manuscript. S.B.T. developed the program, performed the numerical computations, and drafted the manuscript. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENT

This research was funded by the Russian Science Foundation [Grant Number 23-13-00011].

REFERENCES

1. Varfolomeev, S. D.; Bykov, V. I.; Tsybenova, S. B. Kinetic modeling of dynamic processes in the cholinergic synapse. *Russ. Chem. Bull.* **2020**, *69*, 1585–1593. DOI: 10.1007/s11172-020-2939-8.
2. Varfolomeev, S. D.; Bykov, V. I.; Tsybenova, S. B. Kinetics of chemical processes in the human brain. The cholinergic synapse – mechanisms of functioning and control methods. *Dokl. Biochem. Biophys.* **2020**, *492*, 147–151. DOI: 10.1134/S1607672920030126.
3. Varfolomeev, S. D.; Bykov, V. I.; Tsybenova, S. B. Kinetics of chemical processes in the human brain. Proton blockade of acetylcholinesterase and pH-impulse in the mechanism of functioning of the cholinergic synapse. *Dokl. Biochem. Biophys.* **2020**, *491*, 85–88. DOI: 10.1134/S1607672920020155.
4. Varfolomeev, S. D. Molecular basis of intellect: Proton mechanism of recording information. *Moscow Univ. Chem. Bull.* **2022**, *77*, 38–41. DOI: 10.3103/S0027131422010072.
5. Yang, S.; Datta, D.; Woo, E.; Duque, A.; Morozov, Y. M.; Arellano, J.; Slisher, B. S.; Wang, M.; Arnsten, A. F. T. Inhibition of glutamate-carboxypeptidase-II in dorsolateral prefrontal cortex: potential therapeutic target for neuroinflammatory cognitive disorders. *Mol. Psychiatry*. **2022**, DOI: 10.1038/s41380-022-01656-x.
6. Moffett, J. R.; Ariyannur, P.; Arun, P.; Namboodiri, A. M. A. N-Acetylaspartate and N-Acetylaspartylglutamate in central nervous system health and disease. In *Magnetic Res-*

onance Spectroscopy. Stagg, C., Rothman, D., Eds.; Academic Press, **2014**; pp 71–90. DOI: 10.1016/B978-0-12-401688-0.00006-9.

7. Bratek, E.; Ziembowicz, A.; Salinska, E. N-Acetylaspartylglutamate (NAAG) pretreatment reduces hypoxic-ischemic brain damage and oxidative stress in neonatal rats. *Antioxidants* (Basel). **2020**, *9*(9), 877. DOI: 10.3390/antiox9090877.
8. Jiménez-Espinoza, C.; Marcano Serrano, F.; González-Mora, J. L. N-acetylaspartylglutamate metabolism in the cingulated cortices as a biomarker of the etiology in ASD: A ^{1}H-MRS Model. *Molecules.* **2021**, *26*, 675. DOI: 10.3390/molecules26030675.
9. Planas-Fontánez, T. M.; Dreyfus, C. F.; Saitta, K. S. Reactive astrocytes as therapeutic targets for brain degenerative diseases: roles played by metabotropic glutamate receptors. *Neurochem. Res*. **2020**, *45*(3), 541–550. DOI: 10.1007/s11064-020-02968-6.
10. Rudy, C. C.; Hunsberger, H. C.; Weitzner, D. S.; Reed, M. N. The role of the tripartite glutamatergic synapse in the pathophysiology of Alzheimer's disease. *Aging Dis*. **2015**, *6*(2), 131–148. DOI: 10.14336/AD.2014.0423.
11. Bukke, V. N.; Archana, M.; Villani, R.; Romano, A.D.; Wawrzyniak, A.; Balawender, K.; Orkisz, S.; Beggiato, S.; Serviddio, G.; Cassano, T. The dual role of glutamatergic neurotransmission in Alzheimer's disease: from pathophysiology to pharmacotherapy. *Int. J. Mol. Sci.* **2020**, *21*, 7452. DOI: 10.3390/ijms21207452.
12. Morland, C.; Nordengen, K. N-Acetylaspartyl-glutamate in brain health and disease. *Int. J. Mol. Sci*. **2022**, *23*(3), 1268. DOI: 10.3390/ijms23031268.
13. Jessen, F.; Fingerhut, N.; Sprinkart, A. M.; Kühn, K.; Petrovsky, N.; Maier, W.; Schild, H. H.; Block, W.; Wagner, M.; Träber, F. N-Acetylaspartylglutamate (NAAG) and N-Acetylaspartate (NAA) in patients with schizophrenia. *Schizophr. Bull.* **2013**, *39*(1), 197–205. DOI: 10.1093/schbul/sbr127.
14. Rowland, L. M.; Kontson, K.; West, J.; Edden, R. A.; Zhu, H.; Wijtenburg, S. A.; Holcomb, H. H.; Barker, P. B. In vivo measurements of glutamate, GABA, and NAAG in schizophrenia. *Schizophr. Bull*. **2013**, *39*(5), 1096–1104. DOI: 10.1093/schbul/sbs092.
15. Tsai, S. J. Central N-acetylaspartylglutamate deficit: a possible pathogenesis of schizophrenia. *Med. Sci. Monit*. **2005**, *11*(9), HY39–45.
16. Ublinskiy, M.; Semenova, N.; Lebedeva, I.; Akhadov, T. Dynamic of NAA and BOLD after single short stimulus in motor cortex of schizophrenia patients. *Eur. Psychiatry*. **2017**, *41*, S629−S629. DOI: 10.1016/j.eurpsy.2017.01.1023

17. Neale, J. H.; Olszewski, R. A role for N-Acetylaspartylglutamate (NAAG) and mGluR3 in cognition. *Neurobiol. Learn. Mem*. **2019**, *158*, 9–13. DOI:10.1016/j.nlm.2019.01.006.

18. Zink, C.F.; Barker, P.B.; Sawa, A.; Weinberger, D.R.; Wang, M.; Quillian,H.; Ulrich, W. S.; Chen, Q.; Jaffe, A. E.; Kleinman, J. E.; Hyde, T. M.; Prettyman, G. E.; Giegerich, M.; Carta, K.; van Ginkel, M.; Bigos, K. L. Association of missense mutation in $FOLH_1$ with decreased NAAG levels and impaired working memory circuitry and cognition. *Am. J Psychiatry*. **2020**, *177*(12), 1129–1139. DOI: 10.1176/appi.ajp.2020.19111152.

19. Arnsten, A. F. T.; Wang, M. W. The evolutionary expansion of mGluR3-NAAG-GCPII signaling: relevance to human intelligence and cognitive disorders. *Am. J. Psychiatry*. **2020**, *177*(12), 1103–1106. DOI:10.1176/appi.ajp.2020.20101458.

20. Neale, J. H.; Olszewski, R. T.; Zuo, D.; Janczura, K. J.; Profaci, C. P.; Lavin, K. M.; Madore, J. C.; Bzdega, T. Advances in understanding the peptide neurotransmitter NAAG and appearance of a new member of the NAAG neuropeptide family. *J. Neurochem*. **2011**, *118*, 490–498. DOI:10.1111/j.1471-4159.2011.07338.x.

21. Neale, J. H.; Bzdega, T.; Wroblewska, B. N-Acetylaspartylglutamate: the most abundant peptide neurotransmitter in the mammalian central nervous system. *J. Neurochem*. **2000**, *75*(2), 443–452. DOI: 10.1046/j.1471-4159.2000.0750443.x.

22. Westbrook, G. L.; Mayer, M. L.; Namboodiri M. A.; Neale, J. H. High concentrations of N-acetylaspartylglutamate (NAAG) selectively activate NMDA receptors on mouse spinal cord neurons in cell culture. *J. Neurosci*. **1986**, *6*(11), 3385–3392. DOI: 10.1523/JNEUROSCI.06-11-03385.1986.

23. Khrenova, M. G.; Kots, E. D.; Varfolomeev, S. D.; Lushchekina, S. V.; Nemukhin, A. V. Three faces of N-acetylaspartate: activator, substrate, and inhibitor of human aspartoacylase. *J. Phys. Chem. B*. **2017**, *121*, 9389−9397. DOI:10.1021/acs.jpcb.7b08759.

24. Varfolomeev, S. D.; Kots, E. D.; Khrenova, M. G.; Lushchekina, S. V.; Nemukhin, A. V. Supercomputer technologies for structural-kinetic study of mechanisms of enzyme catalysis: A quantum-chemical description of aspartoacylase catalysis. *Dokl. Phys. Chem*. **2017**, *474*, 89−92. DOI: 10.1134/S0012501617060045.

25. Kots, E. D.; Lushchekina, S. V.; Varfolomeev, S. D.; Nemukhin, A. V. Role of protein dimeric interface in allosteric inhibition of N-acetyl-aspartate hydrolysis by human aspartoacylase. *J. Chem. Inf. Model*. **2017**, *57*, 1999−2008. DOI: 10.1021/acs.jcim.7b00133.

26. Kots, E. D.; Khrenova, M. G.; Lushchekina, S. V.; Varfolomeev, S. D.; Grigorenko, B. L.; Nemukhin, A. V. Modeling the complete catalytic cycle of aspartoacylase. *J. Phys. Chem. B*. **2016**, *120*, 4221−4231. DOI: 10.1021/acs.jpcb.6b02542.

27. Varfolomeev, S. D., Gurevich, K. G. *Biokinetics*, Fair-Press, Moscow. 1999.

28. Kots, E. D.; Khrenova, M. G.; Nemukhin, A. V.; Varfolomeev, S. D. Aspartoacylase: a central nervous system enzyme. Structure, catalytic activity and regulation mechanisms. *Russ. Chem. Rev*. **2019**, *88*, 4221−4231. DOI: 10.1070/RCR4842.

29. Ip, I. B.; Berrington, A.; Hess, A. T.; Parker, A. J.; Emir, U. E.; Bridge, H. Combined fMRI-MRS acquires simultaneous glutamate and BOLD-fMRI signals in the human brain. *Neuroimage*. **2017**, *155*, 113–119. DOI: 10.1016/j.neuroimage.2017.04.030.

30. Baslow, M. H.; Guilfoyle, D. N. Evidence that N-acetylaspartylglutamate is the astrocyte-targeted neurovascular coupling agent that regulates slow tonic control of brain blood flow. *J. Clycomics and Metabolism*. **2016**, *1*(1), 25–36 DOI: 10.14302/issn.2572-5424.jgm-16-1028.

31. Varfolomeev, S. D.; Bykov, V. I.; Semenova, N. A.; Tsybenova, S. B. Dynamics of the multipathway regulation of the vasodilator bold effect induced by a nerve impulse: A kinetic model of the neurovascular coupling process. *ACS Chem. Neurosci.* **2021**, *12*, 2202–2208. DOI: 10.1021/acschemneuro.9b00671.

32. Varfolomeev, S. D.; Semenova, N. A.; Bykov, V. I.; Tsybenova, S. B. Kinetic modeling of the blood oxygenation level dependent (BOLD) signals and biocatalytic reactions observed in the human brain using MRI: An analysis of normal and pathological conditions. *ACS Chem. Neurosci*. **2020**, *11*(5), 763–771. DOI: 10.1021/acschemneuro.9b00671.

33. Varfolomeev, S. D.; Semenova, N. A.; Ublinskiy, M. V.; Bykov, V. I.; Tsybenova, S. B. fMRI and MR-spectroscopy in research on triggering and autostabilization of N-acetylaspartate. *Chem. Phys. Lett*. **2019**, *729*, 84–91. DOI: 10.1016/j.cplett.2019.05.033.

34. Varfolomeev, S. D.; Semenova, N. A.; Bykov, V. I.; Tsybenova, S. B. Kinetics of chemical processes in the human brain: Modeling of the BOLD fMRI signal. *Dokl. Phys. Chem*. **2019**, *488*, 39−43. DOI: 10.31857/S0869-56524882157-161.

35. Varfolomeev, S. D.; Semenova, N. A.; Bykov, V. I.; Tsybenova, S. B. Kinetics of chemical processes in the human brain. Trigger effect and self-stabilization of N-acetylaspartic acid. *Dokl. Phys. Chem*. **2019**, *484*, 441−446. DOI: 10.31857/S0869-56524844441-446.

36. Berezin, I. V.; Varfolomeev, S. D.; Martinek, K. Cis-trans isomerization of cinnamoyl-α-chymotrypsin under the action of light and modeling of molecular mechanisms of visual reception. *Dokl. Akad. Nauk. SSSR*. **1970**, *193*, 932–935.

37. Lorenzo, J.; Vuillaume, R.; Binczak, S.; Jacquir S. Spatiotemporal model of tripartite synapse with perinodal astrocytic process. *J. Comput. Neurosci*. **2020**, *48*(1), 1–20. DOI: 10.1007/s10827-019-00734-4.

38. Lalo, U.; Koh, W.; Lee, C. J.; Pankratov, Y. The tripartite glutamatergic synapse. *Neuropharmacol*. **2021**, *199*, 108758. DOI: 10.1016/j.neuropharm.2021.108758.

39. Baslow, M. H.; Guilfoyle, D. N. The bimodal nature of neurovascular coupling: slow tonic and rapid phasic responses are separately controlled by specific astrocyte metabotropic and ionotropic glutamate receptors. *J. Mol. Genet. Med.* **2017**, *11*, 2. DOI: 10.4172/1747-0862.1000266.

40. Baslow, M. H.; Guilfoyle, D. N. Functions of N-acetylaspartate and N-acetylaspartylglutamate in brain: evidence of a role in maintenance of higher brain integrative activities of information processing and cognition. *Adv. Exp. Med. Biol*. **2006**, *576*, 95–112. DOI: 10.1007/0-387-30172-0_7.

41. Baslow, M. H. The vertebrate brain, evidence of its modular organization and operating system: insights into the brain's basic units of structure, function, and operation and how they influence neuronal signaling and behavior. *Front. Behav. Neurosci.* **2011**, *5*, 1–7. DOI: 10.3389/fnbeh.2011.00005.

42. Baslow, M. H. Functions of N-Acetyl-L-Aspartate and N-Acetyl-LAspartylglutamate in the vertebrate brain: role in glial cell-specific signaling. *J. Neurochem*. **2000**, *75*(2), 453–459. DOI: 10.1046/j.1471-4159.2000.0750453.x.

43. Neale, J. H. N-Acetylaspartylglutamate is an agonist at mGluR3 in vivo and in vitro. *J. Neurochem*. **2011**, *119*, 891–895. DOI: 10.1111/j.1471-4159.2011.07380.x.

44. Niswender, C. M.; Conn, P. J. Metabotropic glutamate receptors: physiology, pharmacology, and disease. *Annu. Rev. Pharmacol. Toxicol*. **2010**, *50*, 295–322. DOI:10.1146/annurev.pharmtox.011008.145533.

45. Bradley, S. J.; Challis, R. A. G protein-coupled receptor signalling in astrocytes in health and disease: A focus on metabotropic glutamate receptors. *Biochem. Pharmacol*. **2012**, 84, 249–259. DOI: 10.1016/j.bcp.2012.04.009.

46. Crupi, R.; Impellizzeri, D.; Cuzzocrea, S. Role of metabotropic glutamate receptors in neurological disorders. *Front. Mol. Neurosci.* **2019**, *12*, 20. DOI:10.3389/fnmol.2019.00020.

47. Bodzeta, A.; Scheefhals, N.; MacGillavry, H. D. Membrane trafficking and positioning of mGluRs at presynaptic and postsynaptic sites of excitatory synapses. *Neuropharmacol.* **2021**, *200*, 108799. DOI: 10.1016/j.neuropharm.2021.108799.

48. Li, K.; Lu, M.; Cui, M.; Wang, X.; Zheng Y. The regulatory role of NAAG-mGluR3 signaling on cortical synaptic plasticity after hypoxic ischemia. *Cell Commun. Signal.* **2022**, *20*, 55. DOI: 10.1186/s12964-022-00866-8.

49. Jin, L. E.; Wang, M.; Galvin, V. C.; Lightbourne, T. C.; Conn, P. J.; Arnsten, A. F. T.; Paspalas, C. D. mGluR2 versus mGluR3 metabotropic glutamate receptors in primate dorsolateral prefrontal cortex: Postsynaptic mGluR3 strengthen working memory networks. *Cerebral Cortex*, **2018**, *28*, 974–987. DOI: 10.1093/cercor/bhx005.

50. Sácha, P.; Zámecník, J.; Barinka, C.; Hlouchová, K.; Vícha, A.; Mlcochová, P.; Hilgert, I.; Eckschlager, T.; Konvalinka, J. Expression of glutamate carboxypeptidase II in human brain. *Neurosci.* **2007**, *144*(4), 1361–1372. DOI: 10.1016/j.neuroscience.2006.10.022.

51. Neale, J. H.; Yamamoto, T. N-acetylaspartylglutamate (NAAG) and glutamate carboxypeptidase II: An abundant peptide neurotransmitter-enzyme system with multiple clinical applications. *Progress in Neurobiol.* **2020**, *184*, 101722. DOI: 10.1016/j.pneurobio.2019.101722.

52. Datta, D.; Leslie, S. N.; Woo, E.; Amancharla, N.; Elmansy, A.; Lepe, M.; Mecca, A. P.; Slusher, B. S.; Nairn, A. C.; Arnsten, A. F. T. Glutamate carboxypeptidase II in aging rat prefrontal cortex impairs working memory performance. *Front. Aging Neurosci.* **2021**, *13*, 760270. DOI: 10.3389/fnagi.2021.760270.

53. Armbruster, M.; Dulla, C. G.; Diamond, J. S. Effects of fluorescent glutamate indicators on neurotransmitter diffusion and uptake. *eLife*, **2020**, *9*, e54441. DOI: 10.7554/eLife.54441.

54. Clements, J. D.; Lester, R. A.; Tong, G.; Jahr, C. E.; Westbrook, G. L. The time course of glutamate in the synaptic cleft. *Science*. **1992**, *258*(5087), 1498–501. DOI: 10.1126/science.1359647.

55. Uno, Y.; Coyle, J. T. Glutamate hypothesis in schizophrenia. *Psychiatry Clin. Neurosci.* **2019**, *73*, 204–215. DOI: 10.1111/pcn.12823.

56. Schaller, B.; Mekle, R.; Xin, L.; Kunz, N.; Gruetter, R. Net increase of lactate and glutamate concentration in activated human visual cortex detected with magnetic resonance spectroscopy at 7 Tesla. *J. Neurosci. Res*. **2013**, *91*, 1076–1083. DOI: 10.1002/jnr.23194.

57. Egerton, A.; Grace, A. A.; Stone, J.; Bossong, M. G.; Sand, M.; McGuire, P. Glutamate in schizophrenia: Neurodevelopmental perspectives and drug development. *Schizophr. Res*. **2020**, *223*, 59–70. DOI: 10.1016/j.schres.2020.09.013.

58. Saini, S.; Mancuso, S.; Mostaid, M. S.; Liu, C.; Pantelis, C.; Everall, I.; Bousman, C. Meta-analysis supports GWAS-implicated link between GRM3 and schizophrenia risk. *Transl. Psychiatry*. **2017**, *7*, e1196. DOI: 10.1038/tp.2017.172.

59. Egan, M. F.; Straub, R. E.; Goldberg, T. E.; Yakub, I.; Callicott, J. H.; Hariri, A. R.; Mattay, V. S.; Bertolino, A.; Hyde, T. M.; Shannon-Weickert, C. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. *Proc. Natl. Acad. Sci. USA*. **2004**, *101*, 12604–12609. DOI: 10.1073/pnas.0405077101.

60. Cartmell, J.; Monn, J. A.; Schoepp, D. D. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. *J. Pharmacol. Exp. Ther.* **1999**, *291*(1), 161–170.

61. Rorick-Kehn, L. M.; Johnson, B. G.; Knitowski, K. M.; Salhoff, C. R.; Witkin, J. M.; Perry, K. W.; Griffey, K. I.; Tizzano, J. P.; Monn, J. A.; McKinzie, D. L. In vivo pharmacological characterization of the structurally novel, potent, selective mGlu2/3 receptor agonist LY404039 in animal models of psychiatric disorders. *Psychopharmacol*. **2007**, *193*, 121–136. DOI: 10.1007/s00213-007-0758-3.

62. Olszewski, R. T.; Bukhari, N.; Zhou, J.; Kozikowski, A. P.; Wroblewski, J. T.; Shamimi-Noori, S.; Wroblewska, B.; Bzdega, T.; Vicini, S.; Barton, F. B. NAAG peptidase inhibition reduces locomotor activity and some stereotypes in the PCP model of schizophrenia via group II mGluR. *J. Neurochem*. **2004**, *89*, 876–885. DOI: 10.1111/j.1471-4159.2004.02358.x.

63. Feng, J.-F.; Van, K. C.; Gurkoff, G. G.; Kopriva, C.; Olszewski, R. T.; Song, M.; Sun, S.; Xu, M.; Neale, J. H.; Yuen, P.-W. Post-injury administration of NAAG peptidase inhibitor prodrug, PGI-02776, in experimental TBI. *Brain Res*. **2011**, *1395*, 62–73. DOI: 10.1016/j.brainres.2011.04.022.

64. Zhong, C.; Zhao, X.; Sarva, J.; Kozikowski, A.; Neale, J. H.; Lyeth, B. G. NAAG peptidase inhibitor reduces acute neuronal degeneration and astrocyte damage following lat-

eral fluid percussion TBI in rats. *J. Neurotrauma*. **2005**, *22*, 266–276. DOI: 10.1089/neu.2005.22.266.

65. Feng, J.-F.; Gurkoff, G. G.; Van, K. C.; Song, M.; Lowe, D. A.; Zhou, J.; Lyeth, B. G. NAAG peptidase inhibitor reduces cellular damage in a model of TBI with secondary hypoxia. *Brain Res*. **2012**, *1469*, 144–152. DOI: 10.1016/j.brainres.2012.06.021.

66. Green, J. L.; Dos Santos, W. F.; Fontana, A. C. K. Role of glutamate excitotoxicity and glutamate transporter EAAT2 in epilepsy: Opportunities for novel therapeutics development. *Biochem. Pharmacol*. **2021**, *193*, 114786. DOI: 10.1016/j.bcp.2021.114786.

67. Sarlo, G. L.; Holton, K. F. Brain concentrations of glutamate and GABA in human epilepsy: A review. *Seizure*. **2021**, *91*, 213–227. DOI: 10.1016/j.seizure.2021.06.028.

68. Celli, R.; Santolini, I.; Van Luijtelaar, G.; Ngomba, R. T.; Bruno, V.; Nicoletti, F. Targeting metabotropic glutamate receptors in the treatment of epilepsy: Rationale and current status. *Expert Opin. Ther. Targets*. **2019**, *23*, 341–351. DOI: 10.1080/14728222.2019.1586885.

69. Witkin, J. M.; Gasior, M.; Schad, C.; Zapata, A.; Shippenberg, T.; Hartman, T.; Slusher, B. S. NAALADase (GCP II) inhibition prevents cocaine-kindled seizures. *Neuropharmacol.* **2002**, *43*, 348–356. DOI: 10.1016/s0028-3908(02)00124-7.