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Changes in the Glutamate/GABA System in the Hippocampus of Rats with Age and during Alzheimer's Disease Signs Development

Alena O. Burnyasheva[1], Natalia A. Stefanova[1], Nataliya G. Kolosova[1,a]*, and Darya V. Telegina[1]

[1] *Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, 630090 Novosibirsk, Russia*
[a] *e-mail: kolosova@bionet.nsc.ru*

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Abstract—GABA and glutamate are the most abundant neurotransmitters in the CNS and play a pivotal part in synaptic stability/plasticity. Glutamate and GABA homeostasis is important for healthy aging and reducing the risk of various neurological diseases, while long-term imbalance can contribute to the development of neurodegenerative disorders, including Alzheimer's disease (AD). Normalization of the homeostasis has been discussed as a promising strategy for prevention and/or treatment of AD, however, data on the changes in the GABAergic and glutamatergic systems with age, as well as on the dynamics of AD development, are limited. It is not clear whether imbalance of the excitatory/inhibitory systems is the cause or the consequence of the disease development. Here we analyzed the age-related alterations of the levels of glutamate, GABA, as well as enzymes that synthesize them (glutaminase, glutamine synthetase, GABA-T, and GAD67), transporters (GLAST, GLT-1, and GAT1), and relevant receptors (GluA1, NMDAR1, NMDA2B, and GABAAr1) in the whole hippocampus of the Wistar rats and of the senescence-accelerated OXYS rats, a model of the most common (> 95%) sporadic AD. Our results suggest that there is a decline in glutamate and GABA signaling with age in hippocampus of the both rat strains. However, we have not identified significant changes or compensatory enhancements in this system in the hippocampus of OXYS rats during the development of neurodegenerative processes that are characteristic of AD.

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INTRODUCTION

According to the World Health Organization, Alzheimer's disease (AD) becomes the major cause of senile dementia, with its incidence increasing against the background of longer life expectancy and aging of population in the developed and developing countries [1]. AD is manifested by the pronounced decline in cognitive capacities against the background of accumulation of toxic forms of amyloid-β peptide in the brain, formation of amyloid plaques and neurofibrillary tangles, synaptic failure, and neuronal death [2-4]. Molecular mechanisms underlying AD development are unclear; there are no efficient approaches that can slow down or stop progression of this disease. It is supposed that the age-related changes in the balance of neurotransmitters, i.e., the excitatory glutamatergic and inhibitory GABAergic systems in the brain, can be a prerequisite for the development of AD and make a significant contribution to its progression [5]. Glutamate and gamma-aminobutyric acid (GABA) control many processes in the CNS, including total excitation level in the brain.

Abbreviations: AD, Alzheimer's disease; GABA, gamma aminobutyric acid; GABAAR1, GABA-A receptor subunit 1; GABA-T, GABA transaminase; GAD, glutamic acid decarboxylase (glutamate decarboxylase); GAD67, glutamic acid decarboxylase isoform; GAT1, type 1 GABA transporter; GLAST, glial glutamate and aspartate transporter; GLT-1, glial glutamate transporter 1; GluA1, AMPA receptor subunit 1; NMDAR1, NMDA receptor subunit 1; NMDAR2B, NMDA receptor subunit 2B.

* To whom correspondence should be addressed.

For example, excessive excitation of the inhibitory GABAergic system suppresses and excessive activity of the excitatory glutamatergic system causes excitotoxicity. Balanced interaction between these two neurotransmitters is necessary for physiological homeostasis, whereas long-term imbalance can promote development of mental and neurodegenerative disorders, including AD [6]. Elimination of the excitatory/inhibitory imbalance is actively discussed as a promising strategy for AD prevention and/or treatment [7]; at the same time, the data on age-related changes in the GABAergic and glutamatergic systems, the more so at different stages of AD development, are extremely limited. It remains unclear whether imbalance of the excitatory/inhibitory systems is a cause or a consequence of the AD development. This is associated, first of all, with impossibility to study early preclinical stages of this disease in humans and with lack of its adequate models.

The present study was aimed at assessing contribution of the age-related changes in the balance between the glutamatergic and GABAergic systems in the development and progression of the most widespread (>95% of cases) sporadic form of AD. The work was carried out in the prematurely aging OXYS rats: a unique model of the sporadic form of AD. In these animals, all of the key signs of AD develop spontaneously in the absence of mutations in the *Psen1*, *App*, and *Psen2* genes typical of hereditary AD [8-10]. Already at the age of 3-5 months, OXYS rats show behavioral disorders and decline in cognitive functions, tau protein hyperphosphorylation, impairment of long-term post-tetanic potentiation, synaptic failure, and destructive changes in neurons, which occur against the background of the increased level of amyloid precursor protein (APP), enhanced accumulation of β-amyloid, and formation of amyloid plaques in the brain by the age of 12 months, and reach the clearly marked stages of AD-like pathology by the age of 16-18 months [10, 11]. Previously we have studied age-related changes in the glutamate/GABA system in the retina of OXYS rats and assessed their potential contribution to the development of retinopathy typical of rats [12]. The present work was aimed at comparing the age-related changes in the glutamate/GABA system in the hippocampus of Wistar rats (control) and OXYS rats at different stages of the development of AD signs, including preclinical stage. To this effect, we studied the glutamate and GABA levels in the hippocampus, as well as the levels of key enzymes regulating the glutamate/GABA cycle: glutaminase catalyzing formation of glutamate from glutamine; glutamine synthase catalyzing synthesis of glutamine from glutamate; glutamate decarboxylase (GAD67) converting glutamate to GABA; and the enzyme for GABA degradation: GABA transaminase (GABA-T). In addition, we studied the levels of glutamate and GABA receptors: subunit 1 of the NMDA receptor (NMDAR1) and subunit 2B of the receptor (NMDAR2B), subunit 1 of the AMPA receptor (GluA1) and subunit α1 of the GABA-A receptor (GABAAR1), as well as glutamate carriers: glial glutamate and aspartate transporter (GLAST, also known as EAAT1), glutamate 1 transporter (GLT-1, also known as EAAT2), and GABA transporter (GAT1). Finally, we compared age-related changes in the level of expression of the genes associated with the glutamate and GABA signaling pathways in the hippocampus of OXYS and Wistar rats.

MATERIALS AND METHODS

Experimental animals. All experiments were carried out using OXYS and Wistar (control) male rats. Animals were kept under the standard conditions of vivarium with light cycle 12-h light/12-h darkness; they received granulated feed and water *ad libitum*.

Enzyme immunoassay (ELISA). Glutamate and GABA levels in the hippocampus of OXYS and Wistar rats aged 1.5, 3, 12, and 18 months ($n = 5$ for each group) were estimated by enzyme immunoassay (EIA) using an ELISA Kit for Glutamic Acid (Glu) CES122Ge and an ELISA Kit for Gamma-Aminobutyric Acid (gABA) CEA900Ge, according to the manufacturer's protocol (Cloud-Clone Corp., USA). ELISA kits were chosen to detect only the levels of free glutamate and GABA. Glutamate and GABA as protein components (bound glutamate and GABA, respectively) were excluded from the assay.

Total protein concentration was determined using a ThermoFisher Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, USA). Concentration was determined by plotting a calibration curve using reference protein provided by the manufacturer.

Western blot assay. Content of the key enzymes for synthesis of GABA and glutamate [glutaminase, glutamine synthase, glutamate decarboxylase (GAD67), and GABA transaminase (GABA-T), transporter proteins for glutamate (GLAST and GLT-1) and GABA (GAT1), glutamate (NMDAR1, NMDAR2B, GluA1) and GABA (GABAAR1) receptor subunits] were determined in the hippocampus of OXYS and Wistar rats aged 1.5, 3, 12, and 18 months ($n = 6$ for each group) by Western blot analysis.

Rats were anesthetized by CO_2 inhalation and decapitated. Hippocampus was extracted on ice and frozen in liquid nitrogen. The samples were stored at −70°C till the moment of use. All stages of protein isolation were carried out on ice at 4°C. Hippocampal samples were homogenized using a RIPA lysis buffer (150 mM NaCl; 50 mM Tris-HCl (pH 7.4); 1% Triton X-100; 0.1% sodium dodecyl sulfate (SDS); 1% sodium deoxycholate (Deoxycholic Acid, Sodium Salt), and 1 mM EDTA) with protease and phosphatase inhibitors (P8340 and P5726-5ML; Sigma-Aldrich, USA). After thorough

homogenization, the protein solution was centrifuged at 12,000*g* for 30 min at 4°C. Supernatant was stored at −20°C. Total protein concentration was determined using a ThermoFisher Pierce™ BCA Protein Assay kit (Thermo Fisher; #23225). Calibration curve was used to determine protein concentration using a reference protein from the kit.

Samples (50 μg of total protein) in a loading buffer (10% SDS; 15% β-mercaptoethanol; 50% glycerol; 0.3 M Tris-HCl (pH 6.8); bromophenol blue) were applied onto the lanes of 8% polyacrylamide gel in Tris-Glycine buffer (1.5 M, pH 8.8), separated by electrophoresis, and transferred onto a nitrocellulose membrane (Bio-Rad, USA), which was then blocked with 5% BSA in PBST (1 h). Next the membranes were incubated for 16 h at 4°C with primary antibodies (anti-glutaminase, anti-glutamate synthase, anti-NMDAR1, AMPA receptor anti-subunit 1 (anti-GluA1), anti-GAD67, anti-GABA-T, GABA-A receptor anti-α1, anti-GLT-1 and anti-GAT1 (ab93434, ab64613, ab109182, ab183797, ab26116, ab152134, ab33299, ab41621 and ab426, respectively; Abcam, USA; dilution of 1 : 1000), anti-GLAST and anti-NMDA2B (PA519709 and 71-8600, Invitrogen, USA; dilution of 1 : 1000). β-Actin (42 kDa; ab6276, Abcam; 1 : 5000) and GAPDH (37 kDa; ab8245, Abcam; 1 : 5000) were used as reference proteins. After washing in PBST, the samples were incubated with anti-mouse and anti-rabbit secondary antibodies (ab150115 and ab96886, respectively; Abcam; 1 : 5000) for 1 h at room temperature. Fluorescence was detected with a ChemiDoc MP Imaging System (Bio-Rad). Fluorescence intensity of the bands was measured with the ImageJ software (NIH, USA).

RNA-Seq analysis. High-throughput RNA sequencing (RNA-Seq) of the hippocampal samples of OXYS and Wistar rats aged 20 days, 5 and 18 months ($n = 3$ for each group) was performed using an Illumina Genome Analyzer IIx instrument at the Genoanalitika, Russia. For each sample, ~40 million reads of 50 nucleotides in length were obtained. The reads were mapped to the reference genome of *Rattus norvegicus* (version Rnor_5.0.76) using the TopHat software (v. 2.0.10). Based on the RNA-Seq data at a significance level of padj < 0.05, lists of the differentially expressed genes were compiled [13, 14].

The list of genes for the glutamatergic signaling pathway was retrieved from the rat genome database (RGD; 126 rat genes; https://rgd.mcw.edu/). The list of genes for the GABAergic synapse was obtained from the KEGG pathway database (89 human genes; https://www.genome.jp/kegg/).

Statistical analysis. Statistical processing of the results was performed with the STATISTICA software package (version 10.0). The Kolmogorov–Smirnov test was used to check normal distribution. The analysis included all values lying within the range of three root mean square deviations from the sample mean. The analysis of variance was used, followed by *post hoc* comparison of intergroup average values using the Newman–Keuls test. "Genotype" and "age" were considered as independent factors. The data are presented as a mean ± standard deviation (M ± SD). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Assessment of glutamate and GABA levels in the hippocampus of OXYS and Wistar rats of different ages. The first stage of the work included enzyme immunoassay (ELISA) of free glutamate and GABA levels in the hippocampus of Wistar and OXYS rats of different age. No any inter-lineage and age-related differences in the levels of glutamate (Fig. 1a) and GABA (Fig. 1b) in the hippocampus of rats of both lineages were revealed. Two-factor analysis of variance (ANOVA) did not show any effects of "genotype" and "age" factors. Thus, the level of neurotransmitters under the study remained stable in the hippocampus of OXYS and Wistar rats throughout their life.

Age-related changes in the glutamatergic system in the hippocampus of Wistar and OXYS rats. When assessing age-related changes in the glutamatergic system in the rat brain, we analyzed expression of the key enzymes for glutamate synthesis and degradation: proteins glutaminase and glutamine synthase, as well as transporter proteins GLAST, GLT-1, and NMDAR1, NMDAR2B, and GluA1 glutamate receptor subunits (AMPA receptor subunits). Glutamate cannot penetrate through the blood–brain barrier; accordingly, glutamate is synthesized in the brain *de novo* in astrocytes and neurons from glutamine with involvement of glutaminase. In astrocytes, glutamate is converted into glutamine with involvement of glutamine synthase [15].

According to the two-way analysis of variance, the levels of glutaminase (Fig. 2a) and glutamine synthase (Fig. 2b) does not depend on the age or genotype of the animals: we have not observed any differences between the levels of these proteins in the hippocampus of OXYS and Wistar rats.

An important factor determining availability of glutamate for signaling processes is the system of its recapture and recycling. Glutamate uptake from the synaptic cleft is necessary for normal neurotransmission in glutamatergic synapses, because high level of extracellular glutamate may have a toxic effect on the neurons and synapses. The GLAST and GLT-1 transporters remove glutamate from the extracellular space, which is necessary to maintain low nontoxic concentrations of this neurotransmitter [16] As assessed with ANOVA, the levels of GLAST and GLT-1 proteins in the hippocampus depended on the "age" factor ($F_{3,37} = 10.2$; $p < 0.001$

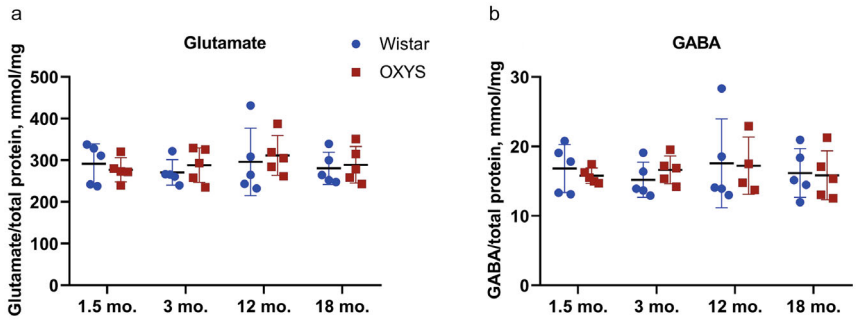

Fig. 1. Levels of glutamate (a) and GABA (b) in the hippocampus of Wistar and OXYS rats of different age. The data are presented as M ± SD (n = 5).

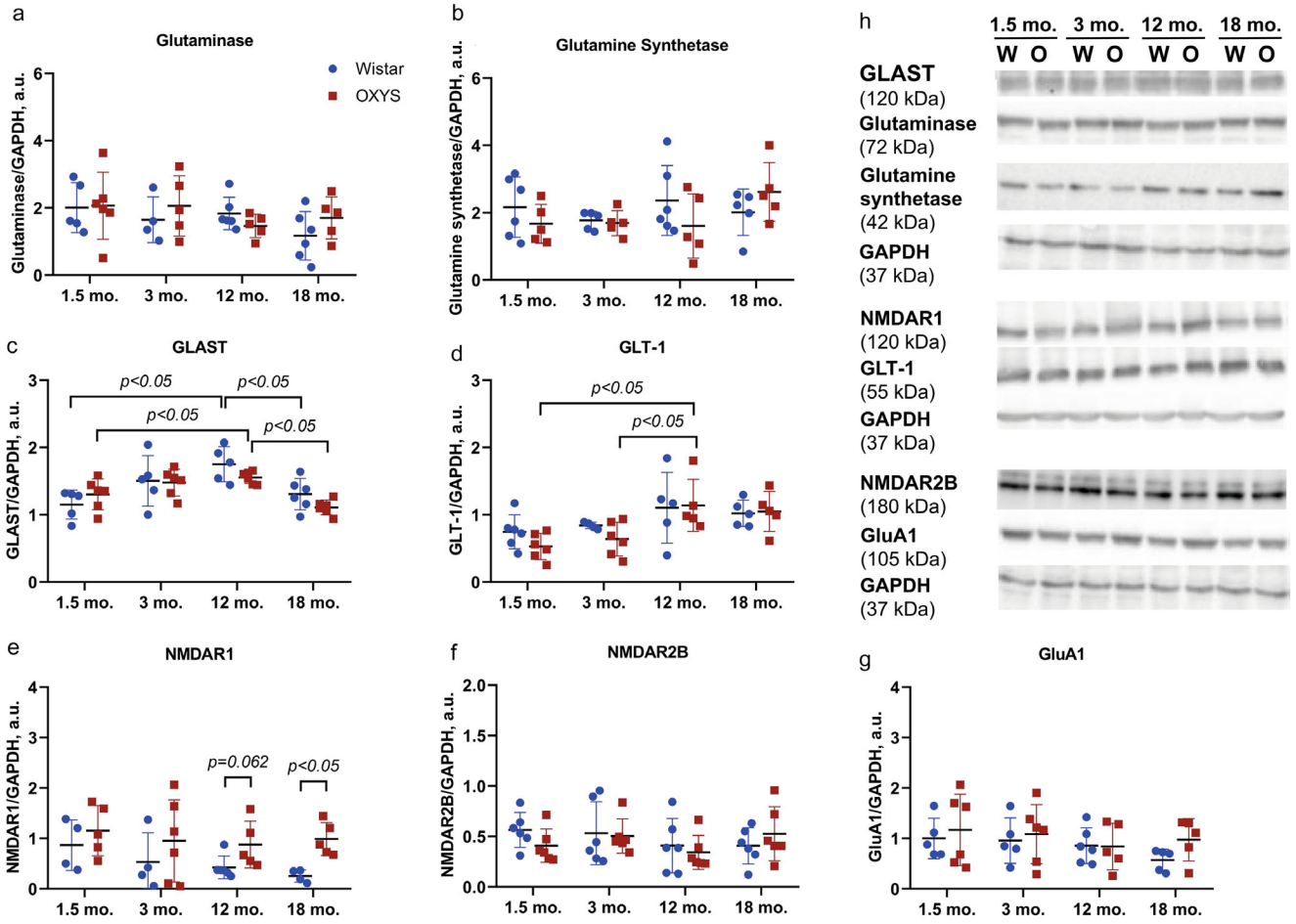

Fig. 2. Age-related changes in the protein levels of glutaminase (a), glutamine synthase (b), GLAST (c), GLT-1 (d), NMDAR1 (e), NMDAR2B (f), GluA1 (g) in the hippocampus of Wistar and OXYS rats. h) Representative images of the Western blot analysis of the proteins: W, Wistar, O, OXYS. The data are presented as M ± SD (n = 4-6).

and $F_{3.31} = 4.4$; $p < 0.01$, respectively) but did not depend on the "genotype" factor. Comparison of the average group values showed that the level of GLAST in the rats of both lineages increased by the age of 12 months and decreased to the level of 1.5-month-old animals by the age of 18 months (Fig. 2c). The level of GLT-1 in the OXYS rats increased from 3 to 12 months and remained at the same level at the age of 18 months, while in the Wistar rats the level of GLT-1 did not significantly change with age (Fig. 2d). There were no inter-lineage differences in the levels of GLAST and GLT-1 transporters.

Postsynaptic terminal recognizes glutamate using glutamate receptors [6]. That is why we analyzed the levels of subunits of the NMDA- and AMPA-type ionotropic glutamate receptors mediating fast neurotransmission [AMPA receptor subunit 1 (also known as GluA1) and NMDA receptor subunits NR1 and NR2B (NMDAR2B and NMDAR1; also known as GluN2B

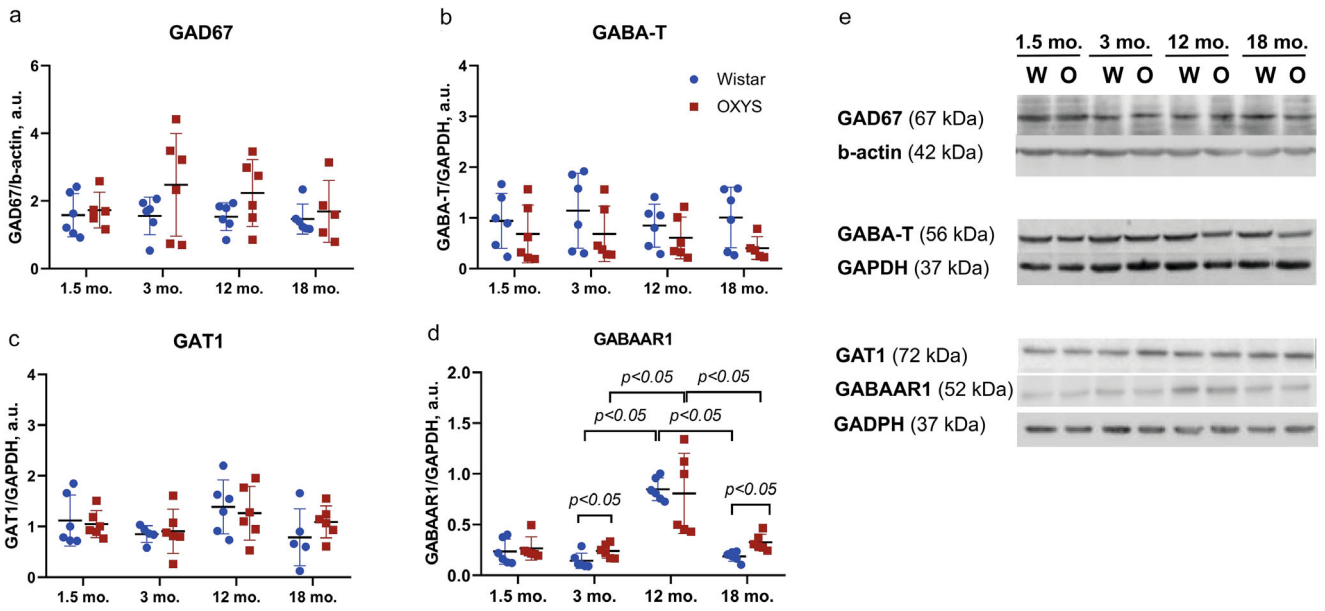

Fig. 3. Age-related changes in the levels of proteins GAD67 (a), GABA-T (b), GAT1 (c), and GABAAR1 (d) in the hippocampus of Wistar and OXYS rats. e) Representative images of the Western blot analysis of the proteins: W, Wistar; O, OXYS. The data are presented as M ± SD (n = 4-6).

and GluN1, respectively)] in the hippocampus of Wistar and OXYS rats of different age. Based on the two-way analysis of variance, the level of NMDAR1 protein depended only on the "age" factor ($F_{3.34} = 1.6$; $p < 0.05$). Comparison of the average group values showed that the level of NMDAR1 decreased with age in the Wistar rats: by the age of 12 months it became reliably lower ($p < 0.05$) compared to the 1.5-month-old animals (Fig. 2e). In the OXYS rats no significant age-related changes were detected; therefore, even at the age of 12 months, they demonstrated an increasing trend of the level of NMDAR1 ($p = 0.06$) in comparison to the Wistar rats; at the age of 18 months in the OXYS rats, the level of NMDAR1 was reliably higher ($p < 0.05$; Fig. 2e). The levels of NMDAR2B and GluA1 in the hippocampus of OXYS and Wistar rats were not different and did not change with age (Fig. 2, f and g).

Age-related changes in the GABAergic system in the hippocampus of Wistar and OXYS rats. Next, we assessed expression of the key enzymes for GABA synthesis (glutamate dexarboxylase, GAD67) and degradation (GABA transaminase, GABA-T). As was shown by the two-way analysis of variance, the level of GAD67 was higher ($F_{1.38} = 4.2$; $p < 0.05$) and the level of GABA-T was lower ($F_{1.39} = 6.3$; $p < 0.02$) in the OXYS rats compared to the Wistar rats; at the same time, age had no effect on the levels of these proteins (Fig. 3, a and b). Age had no effect on the level of GAT1: the transporter that removes GABA from the synaptic cleft. Differences in the GAT1 level in the hippocampus of OXYS and Wistar rats were not detected either (Fig. 3c).

The level of GABAAR1 depended on the age of animals ($F_{3.40} = 39.7$; $p < 0.001$) but was independent of their genotype. The level of GABAAR1 significantly increased by the age of 12 months and then decreased by the age of 18 months in the rats of both lineages ($p < 0.05$; Fig. 3d). At the same time, comparison of the average group values showed that the level of GABAAR1 at the age of 3 and 18 months in the OXYS rats was higher than in the Wistar rats ($p < 0.05$).

Age-related changes in the expression of the genes associated with the glutamate and GABA signaling pathways in the hippocampus of OXYS and Wistar rats. For assessing the changes in expression of the genes associated with glutamatergic and GABAergic synapses in the OXYS rats, we analyzed the previous data obtained by RNA-Seq of the hippocampus of 20-day-old, 5- and 18-month-old OXYS and Wistar rats [13, 14]. In the OXYS rats, expression of 29 out of 126 genes associated (according to RGD) with glutamatergic synapse changed from the age of 20 days to the age of 5 months, and expression of 56 genes changed from 5 to 18 months. In the Wistar rats, expression of 34 genes changed from the age of 20 days to the age of 5 months and expression of 51 genes changed from 5 to 18 months. Out of the 88 genes associated (according to the KEGG pathway database) with the GABAergic synapse, expression of 13 genes changed from the age of 20 days to 5 months and expression of 41 genes changed from 5 to 18 months in the hippocampus of OXYS rats; in the Wistar rats, expression of 21 genes changed from the age of 20 days to 5 months and expression of 37 genes changed from the age of 5 to 18 months. It should be noted that expression of the vast majority of the genes associated with the glutamate/GABA signaling pathways decreased at the age from 5 to 18 months in the hippocampus of rats from both lineages (table).

Age-related changes in expression of the genes associated with the glutamate/GABA signaling pathways in the hippocampus of OXYS and Wistar rats

Gene name	Gene symbol	20 days-5 months		5-18 months	
		OXYS	Wistar	OXYS	Wistar
4-Aminobutyrate aminotransferase	*Abat*			↓	↓
Adenylate cyclase 1	*Adcy1*		↑	↓	↓
Adenylate cyclase 2	*Adcy2*		↑	↓	↓
Adenylate cyclase 4	*Adcy4*		↓		↑
Adenylate cyclase 5	*Adcy5*			↓	↓
Adenylate cyclase 6	*Adcy6*	↓	↓		
Adenylate cyclase 9	*Adcy9*	↑	↑	↓	↓
Voltage-gated N-type calcium channel subunitalpha-1B	*Cacna1b*			↓	
Voltage-gated N-type calcium channel subunit alpha-1C	*Cacna1c*			↓	↓
Calcineurin-like EF-hand protein 1	*Chp1*			↓	↓
Discs large MAGUK scaffold protein 4	*Dlg4*			↓	↓
DLG associated protein 1	*Dlgap1*	↑	↑	↓	↓
Type 1 protein associated with type A GABA receptor	*Gabarapl1*		↑		
Type 2 protein associated with type A GABA receptor	*Gabarapl2*			↑	↑
Type B GABA receptor subunit 1	*Gabbr1*			↑	
Type B GABA receptor subunit 2	*Gabbr2*			↓	↓
Type A GABA receptor subunit alpha 1	*Gabra1*			↓	↓
Type A GABA receptor subunit alpha 2	*Gabra2*			↓	↓
Type A GABA receptor subunit alpha 3	*Gabra3*			↓	↓
Type A GABA receptor subunit alpha 5	*Gabra5*			↓	↓
Type A GABA receptor subunit beta 1	*Gabrb1*	↑	↑	↓	↓
Type A GABA receptor subunit beta 2	*Gabrb2*			↓	↓
Type A GABA receptor subunit beta 3	*Gabrb3*			↓	↓
Type A GABA receptor subunit delta	*Gabrd*	↑	↑	↑	
Type A GABA receptor subunit gamma 2	*Gabrg2*	↑		↓	↓
Type A GABA receptor subunit gamma 3	*Gabrg3*				↓
Type A GABA receptor subunit theta	*Gabrq*				↓
Glutamate decaboxylase 2	*Gad2*			↓	↓

Table (*cont.*)

Gene name	Gene symbol	20 days-5 months		5-18 months	
		OXYS	Wistar	OXYS	Wistar
Glutaminase	*Gls*			↓	↓
Glutaminase 2	*Gls2*		↑		
Subunit G protein subunit alpha i1	*Gnai1*			↓	↓
Gprotein subunitalpha i3	*Gnai3*		↓	↓	
G protein subunit alpha o1	*Gnao1*			↓	↓
G protein subunit alpha q	*Gnaq*			↓	↓
Complex locus GNAS	*Gnas*			↑	↑
G protein subunit beta 1	*Gnb1*			↓	↓
G protein subunit beta 2	*Gnb2*			↑	
G protein subunit beta 4	*Gnb4*	↓	↓	↓	↓
G protein subunit beta 5	*Gnb5*		↑		
G protein subunit gamma 12	*Gng12*	↓	↓	↓	↓
G protein subunit gamma 2	*Gng2*	↓		↓	↓
G protein subunit gamma 3	*Gng3*	↓		↑	
G protein subunit gamma 4	*Gng4*	↓			↓
G protein subunit gamma 5	*Gng5*			↑	↑
G protein subunit gamma 8	*Gng8*				↑
Glutamate ionotropic receptor AMPA type subunit 1	*Gria1*		↑	↓	↓
Glutamate ionotropic receptor AMPA type subunit 3	*Gria3*			↓	
Glutamate ionotropic receptor kainite type subunit 2	*Grik2*			↓	
Glutamate ionotropic receptor kainite type subunit 3	*Grik3*			↓	↓
Glutamate ionotropic receptor kainite type subunit 4	*Grik4*			↑	
Glutamate ionotropic receptor NMDA type subunit 2A	*Grin2a*	↑	↑	↓	↓
Glutamate ionotropic receptor NMDA type subunit 2B	*Grin2b*	↑	↑		↓
Glutamate ionotropic receptor NMDA type subunit 2D	*Grin2d*				↓
Glutamate ionotropic receptor NMDA type subunit 3A	*Grin3a*			↓	↓
Glutamate ionotropic receptor NMDA type subunit 3B	*Grin3b*				↑
Glutamate metabotropic receptor 1	*Grm1*	↑	↑	↓	↓

Gene name	Gene symbol	20 days-5 months		5-18 months	
		OXYS	Wistar	OXYS	Wistar
Glutamate metabotropic receptor 2	*Grm2*		↓		
Glutamate metabotropic receptor 3	*Grm3*	↑	↑	↓	↓
Glutamate metabotropic receptor 4	*Grm4*	↓	↓		
Glutamate 5	*Grm5*			↓	↓
Glutamate metabotropic receptor 7	*Grm7*			↓	↓
Huntingtin-associated protein 1	*Hap1*	↑	↑		
Homer scaffold protein 1	*Homer1*			↓	↓
Homer scaffold protein 2	*Homer2*			↓	↓
Homer scaffold protein 3	*Homer3*			↑	↑
Inositol-1,4,5-triphosphate receptor type 1	*Itpr1*	↑	↑	↓	↓
Inositol-1,4,5-triphosphate receptor type 2	*Itpr2*	↓	↓		
Member 3 of potassium inwardly rectifying channel subfamily J	*Kcnj3*			↓	↓
Member 6 of potassium inwardly rectifying channel subfamily J	*Kcnj6*			↓	
Mitogen-activated protein kinase 1	*Mapk1*	↑	↑	↓	↓
N-ethylmaleimide-sensitive factor, vesicle-fusing ATPase	*Nsf*		↑		
Phospholipase A2, group IIC	*Pla2g2c*		↑		
Phospholipase A2, group III	*Pla2g3*	↓	↓	↑	↑
Phospholipase A2, group IVE	*Pla2g4e*	↓			
Phospholipase A2, group V	*Pla2g5*	↓			
Phospholipase A2, group VI	*Pla2g6*				↓
Phospholipase C beta 1	*Plcb1*	↑		↓	↓
Phospholipase C beta 4	*Plcb4*		↓	↓	
Phospholipase C like 1 (inactive)	*Plcl1*			↓	
Phospholipase D1	*Pld1*	↓	↓	↓	↓
Phospholipase D2	*Pld2*	↓	↓		
Protein phosphatase 3, catalytic subunit alpha	*Ppp3ca*	↑		↓	
Protein phosphatase 3, catalytic subunit beta	*Ppp3cb*			↓	
Protein phosphatase 3, catalytic subunit gamma	*Ppp3cc*				↑

Table (*cont.*)

Gene name	Gene symbol	20 days-5 months		5-18 months	
		OXYS	Wistar	OXYS	Wistar
Protein phosphatase 3, regulatory subunit B, alpha	*Ppp3r1*		↑	↓	
cAMP-dependent protein kinase, catalytic subunit beta	*Prkacb*			↓	↓
Protein kinase C alpha	*Prkca*			↓	↓
Protein kinase C beta	*Prkcb*			↓	↓
Protein kinase C gamma	*Prkcg*		↑	↓	
cAMP-dependent protein kinase X-linked catalytic subunit	*Prkx*		↓		
SH3 and multiple ankyrin repeat domains 1	*Shank1*	↑			
Solute carrier family 12 member 5	*Slc12a5*	↑	↑		
Solute carrier family 17 member 6	*Slc17a6*	↓	↓	↓	↓
Solute carrier family 17 member 8	*Slc17a8*		↓	↓	
Solute carrier family 1 member 1	*Slc1a1*			↓	↓
Solute carrier family 1 member 2	*Slc1a2*	↑	↑	↓	↓
Solute carrier family 1 member 3	*Slc1a3*	↓			
Solute carrier family 1 member 7	*Slc1a7*	↓	↓		
Solute carrier family 38 member 1	*Slc38a1*			↓	↓
Solute carrier family 1 member 2	*Slc38a2*			↓	
Solute carrier family 38 member 3	*Slc38a3*	↓	↓		
Solute carrier family 6 member 1	*Slc6a1*				↓
Solute carrier family 6 member 11	*Slc6a11*		↑	↓	↓
Solute carrier family 6 member 1	*Slc6a12*		↓		
Solute carrier family 6 member 13	*Slc6a13*		↓		
Motor protein kinesin 2	*Trak2*			↓	↓
Member 1 of subfamily C of transient receptor cation potential channels	*Trpc1*			↓	↓

Note. Upward pointing arrow, genes with the age-related increase in expression; downward pointing arrow, genes with the age-related decrease in expression.

At the age of 20 days, expression of only four genes associated with the glutamatergic synapse in the hippocampus of the OXYS rats was different from that in the Wistar rats: the mRNA levels of *Grin3b*, *Grm6*, and *Slc1a2* were increased and that of *Pla2g5* was decreased (padj < 0.05). In the OXYS rats aged 5 months, expression of the *Grin3b* and *Pla2g2d* was changed (increased); at the age of 18 months, expression of the *Pla2g6* gene was increased and that of the *Gng12*, *Grm6*, *Ppp3r1*, and *Slc1a1* genes was decreased. Among the genes

associated with the GABAergic synapse, only at the age of 18 months we identified differentially expressed genes with reduced expression in the OXYS rats: *Gad2*, *Gng12*, *Plcl1*, and *Trak2*.

Thus, we have failed to detect any significant interlineage differences in the level of expression of the genes associated with glutamatergic and GABAergic synapses. With age, the hippocampus of rats of both lineages showed, in general, similar changes in the expression of the genes associated with glutamate and GABA signaling pathways; at the same time, the mRNA level of the vast majority of these genes showed a regular decrease in both Wistar and OXYS rats.

DISCUSSION

Cox et al. [5] consider age-related decline in the efficiency of glutamatergic signal transduction, which has been found in different animal species, as one of the conservative manifestations of aging, similar to sarcopenia or osteoporosis. Naturally, decline and impairment of synaptic plasticity is accelerated in the case of premature aging, including AD. The main goal of the present study was to assess contribution of the age-related changes in the glutamatergic and GABAergic systems to the emergence and progression of the signs of AD in the brain of OXYS rats, the model of its sporadic form. Unexpectedly, we have not revealed significant differences between functioning of these systems in the hippocampus of OXYS rats and control Wistar rats. One of the possible causes was the decrease in total protein level used to normalize the levels of glutamate and GABA in the present work. In another work, where we studied hippocampal metabolome by NMR spectroscopy, all parameters were normalized to the sample weight and showed increase in the GABA level from the age of 20 days to the age of 5 months and its decrease from the age of 5 months to the age of 18 months in the rats of both lineages, as well as analogous age-related changes in the glutamate level in the OXYS rats [17]. Such results indicate that the age-related changes in the balance of the glutamate/GABA system occur in the hippocampus of rats of both lineages. It should be noted that the published data on changes in the level of glutamate and the enzymes for its synthesis and degradation with age and during AD development are contradictory. Some works report age-related decrease in the level of glutamate in the anterior cingulate gyrus, hippocampus, and other brain regions [18-22]. Other authors state that there are no age-related changes in the glutamate and glutamine synthesis in the rat brain and in the glutamate cycle, as confirmed by the data on the absence of changes in the glutamate synthase activity [23, 24].

The results of the current study have shown that the levels of the key enzymes for glutamate synthesis (neuronal glutaminase) and degradation (glial glutamate synthase) do not change with age and are not different in the OXYS and Wistar rats, indicating stability of its synthesis in the hippocampus of rats throughout their entire life. At the same time, it should be noted that the absence of changes in the glutamate level cannot be unambiguously considered as an indicator of stability of the glutamatergic system in aging and AD development. Glutamate excitotoxicity is mediated mainly by the impairment of its recapture system, which leads to the high level of glutamate in synaptic clefts and, as a consequence, hyperactivation of NMDA receptors [25].

Thus, an important factor that determines availability of glutamate for the signal transduction pathway is the system of its recapture and recycling. Glutamate cannot penetrate through the blood–brain barrier and is produced mainly by neurons and astrocytes. Nevertheless, neurons *per se* are unable to synthesize glutamate from glucose via the tricarboxylic acid cycle due to the absence of pyruvate carboxylase [15]. In view of the above, glutamate formation in astrocytes plays an important role; it occurs through the two pathways: synthesis *de novo* in the tricarboxylic acid cycle (it accounts for ~15% of glutamate) or glutamine "recycling" from GABA and glutamate through recapture of neurotransmitters [6]. Under physiological conditions, astrocytes remove ~90% of all glutamate released by the CNS using excitatory amino acid transporters GLAST and GLT-1 required for maintaining low, nontoxic concentrations of this neurotransmitter [26]. As our study has shown, the level of GLAST in the hippocampus of both Wistar and OXYS rats increased by the age of 12 months and decreased by the age of 18 months. We were unable to find any age-related changes in the level of GLT-1 in the Wistar rats, while in the OXYS rats it increased considerably from the age of 3 to 12 months, which may indicate the change in glutamate recapture. This is probably due to accumulation of the toxic forms of β-amyloid in the hippocampus of OXYS rats by the age of 12 months [9]. It should be noted that insignificant changes in the amount of GLT-1 were also detected in the hippocampus of AD patients: it has been reported that the spatial pattern of expression of this transporter is changed and the increased immunostaining of GLT-1 is observed in the astrocyte processes and in neuropile, especially in the CA1 and CA3 areas of the hippocampus, as well as in the dentate gyrus [27].

The NMDA and AMPA receptors are present in approximately 70% of synapses of the mammalian brain, mainly in the cerebral cortex, amygdaloid body, striated body, and hippocampus. Specific localization of these receptors is of great significance, because glutamatergic system plays an important role not only in neuroplasticity but also in excitotoxicity [28]. It has been shown that the glutamatergic system mediated by NMDA receptors becomes hypofunctional with age, and this deficit

can lead to cognitive dysfunction under both normal and pathological conditions [5]. In addition, there is evidence that the number of NMDA receptors on the postsynaptic terminals of neurons in the hippocampus decreases with age [29, 30], thereby considerably reducing glutamate bioavalability [31]. It is known that NMDA receptors are heterotetramers consisting of two obligatory subunits NMDAR1 and two regulatory subunits GluN2 (A–D) or GluN3 (A or B) localized mainly in the dendrites of neurons. Since the hippocampus is a cerebral area regulating cognitive functions, regulatory subunits are mainly represented by NMDA2A or NMDA2B (GluN2A and GluN2B, respectively) [32].

In the present work, we have assessed the age-related changes in the level of obligate subunit NMDAR1 and subunit NMDA2B mediating excitotoxic effects of glutamate in the rat hippocampus [33]. The NMDAR2B protein level did not change significantly with age and was not different in the Wistar and OXYS rats, while the level of subunit NMDAR1 decreased with age in the hippocampus of Wistar rats but did not change significantly in the OXYS rats. As a result, the level of NMDAR1 became much higher by the age of 18 months in the OXYS rats compared to the Wistar rats. It should be noted that similar changes in the level of NMDAR1 have been found in the AD patients [34]. It is likely that the increased level of NMDAR1 in the hippocampus in the case of AD is a compensatory mechanism, because it has been reported that the increased number of subunits NMDAR1 and NMDA2A, but not of NMDAR2B, is associated with spatial memory consolidation and formation [34].

In the hippocampus, AMPA receptors localized mainly in neurons are in composition of the majority of excitatory synapses, especially in the CA1 region (~80% of all receptors). The best studied AMPA receptor subunit is GluA1 [35]. Moreover, the GluA1-related impairment of synaptic plasticity is considered by many authors as one of the key events at the early stages of AD development [36]. Our analysis of the GluA1 level did not reveal any significant changes with aging and development of the signs of AD in the OXYS rats. Probably, this is due to the fact that we have assessed it in the whole hippocampus, while the changes in the GluA1 expression may be differently regulated in the different areas of this brain structure [34, 35].

Previously it has been considered that the GABAergic neurons are more resistant to the pathological effects of β-amyloid compared to the cholinergic or glutamatergic neurons [36]. The hypothesis that has been put forward in recent years suggests that the excitatory/inhibitory imbalance could cause GABAergic dysfunction, which increases susceptibility of the neurons to unfavorable external factors and pathological stress, contributing to the impairment of functional connections in the brain during the development of AD [37]. In the present study, we have not revealed any differences in the GABA levels in the hippocampus of OXYS and Wistar rats. The only direct precursor of GABA in the CNS is glutamate, which is converted into GABA by glutamic acid decarboxylase, or glutamate decarboxylase (GAD). In the mammalian brain, GAD has two isoforms: GAD65 and GAD67 [38]. GAD65 is mainly localized on the presynaptic nerve endings, while GAD67 is distributed all around a cell. It should be noted that more than 90% of GABA in the brain is synthesized by GAD67 [39, 40]. Mice with the GAD67 gene knockout die within a week after birth; however, mice with the GAD67 expression deficiency are viable, though exhibit abnormal behavior [41]. On the contrary, mice with the GAD65 gene knockout survive but are prone to convulsions [42]. Dysfunction of GAD67 is related to the brain disorders such as schizophrenia [43], bipolar disorder [44] and Parkinson's disease [45]. It has been reported that the expression of GAD67 is unchanged in the post mortem samples of the brain tissues from AD patients; however, at the same time, it is still unclear whether GAD67 is involved in progression of the disease [46]. In addition, it has been shown that age and sex have no effect on the GAD67 expression in the human hippocampus and cerebral cortex [47]. According to our data, in the hippocampus of OXYS rats, the level of GABA-T (enzyme responsible for GABA degradation in the brain and localized mainly in astrocytes) is considerably lower, while the level of GAD67, which catalyzes GABA formation in neurons, is higher than in the Wistar rats. These results indicate the enhanced demand for GABA formation in the hippocampus of OXYS rats. At the same time, we have shown no reliable differences in the content of GABA transporter GAT1, which removes GABA from the synaptic cleft.

Previously, experiments *in vitro* have shown that β-amyloid neurotoxicity reduces activity of the GABAergic neurons and attenuates inhibitory postsynaptic potentials by suppressing postsynaptic GABA receptors [48, 49]. However, in the hippocampus of OXYS rats, the level of postsynaptic GABA receptor GABAAR1 at the age of 3 months (in the period of manifestation of the signs of AD) and in the period of their active progression (12 months) was higher than in the Wistar rats. In the hippocampus, the highest level of GABAAR1 expression is observed in the CA1 area and, according to some studies, its expression does not change with age [50, 51]. We believe that the enhanced GABAAR1 expression in the hippocampus of the one-year-old OXYS rats that we have observed may be due to neurodegenerative changes, which are noted as early as at the age of 3-5 months and progress with aging [52]. There is considerable accumulation of β-amyloid in the brain structures of the OXYS rats by the age of 12 months [9], and we consider it as a possible cause of increase in GABAAR1; however, this assumption needs further experimental verification.

In order to assess age-related changes in the glutamatergic and GABAergic systems and their potential contributions to the development of the signs of AD, we have analyzed gene expression in the hippocampus of OXYS and Wistar rats of different ages using the RNA-Seq data. Our analysis has shown their significant age-related changes in the hippocampus of the rats in both lineages; however, we have failed to find any inter-lineage differences. Thus, the mRNA level of the genes encoding components of the glutamate and GABA signaling pathways are not different in the Wistar and OXYS rats at all stages of development of the signs of AD. The exception that we have noted is the genes encoding glutamate receptors (*Grin3b* and *Grm6*), glutamate decarboxylase 2 (*Gad2*), G protein subunit (*Gng12*), the family of solute carriers (*Slc1a1* and *Slc1a2*), phospholipase A2 (*Pla2g2d*, *Pla2g5*, and *Pla2g6*), phospholipase C (*Plcl1*), protein phosphatase 3 (*Ppp3r1*), and transport protein kinesin 2 (*Trak2*). Nevertheless, the changes in expression of these genes at different stages of neurodegeneration in OXYS rats did not allow us to formulate any hypothesis about contribution of these genes to the pathogenesis of AD. On the contrary, we have revealed a clear age-related decrease in the expression of the genes associated with glutamate/GABA signal transduction in the rats of both lineages.

Generally, our results indicate absence of the changes or compensatory activation in the glutamate- and GABAergic systems with aging and development of the signs of AD in the OXYS rats, which seem to be a consequence of the development of neurodegenerative processes.

CONCLUSIONS

According to Cox et al. [5], the decrease in glutamatergic transmission can be used as a biomarker of transition from physiological to pathological aging. Based on the results of our study, we can conclude that there is a considerable age-related decrease in the glutamate and GABA signal transduction in the hippocampus of Wistar rats; however, in the hippocampus of OXYS rats there are no substantial changes or compensatory increase in this system during the development of neurodegenerative processes typical of AD. The study of AD pathogenesis is complicated by its heterogeneity, various pathophysiological scenarios, and existence of several molecular subtypes of this disease [53]. Further studies are required to detect the changes in the balance of neurotransmitter systems. This knowledge will be an important step on the pathway to personalized medicine for the patients with this neurodegenerative disease.

Contributions. A.O.B. and N.A.S. experimental work; A.O.B., N.A.S., D.V.T., and N.G.K. discussion of research results; A.O.B. and D.V.T. writing the manuscript; N.G.K., N.A.S., and D.V.T. editing the manuscript.

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Ethics declarations. The authors declare no conflict of interest in financial or any other sphere. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All studies were carried out with OXYS and Wistar (control) male rats on the basis of the Center for Collective Use "Gene Pools of Laboratory Animals", Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, in accordance with the ethical standards of European Union Directive 2010/63/EU of September 22, 2010. All experiments were approved and performed in accordance with the guidelines of the Ethics Committee on Animal Trials at the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia (Resolution no. 12000-496 of April 2, 1980).

REFERENCES

1. Wang, R., and Reddy, P. H. (2017) Role of glutamate and NMDA receptors in Alzheimer's disease, *J. Alzheimers Dis.*, **57**, 1041-1048, doi: 10.3233/JAD-160763.
2. Selkoe, D. J., and Hardy, J. (2016) The amyloid hypothesis of Alzheimer's disease at 25 years, *EMBO Mol. Med.*, **8**, 595-608, doi: 10.15252/emmm.201606210.
3. Wyss-Coray, T. (2016) Ageing, neurodegeneration and brain rejuvenation, *Nature*, **539**, 180-186, doi: 10.1038/nature20411.
4. Polanco, J. C., Li, C., Bodea, L. G., Martinez-Marmol, R., Meunier, F. A., and Götz, J. (2017) Amyloid-β and tau complexity – towards improved biomarkers and targeted therapies, *Nat. Rev. Neurol.*, **14**, 22-39, doi: 10.1038/nrneurol.2017.162.
5. Cox, M. F., Hascup, E. R., Bartke, A., and Hascup, K. N. (2022) Friend or foe? Defining the role of glutamate in aging and Alzheimer's disease, *Front. Aging*, **3**, 929474, doi: 10.3389/fragi.2022.929474.
6. Hampe, C. S., Mitoma, H., and Manto, M. (2018) GABA and glutamate: their transmitter role in the CNS and pancreatic islets, in *GABA and Glutamate – New Developments in Neurotransmission Research*, IntechOpen, Book Chapter 5, pp. 65-90, doi: 10.5772/intechopen.70958.
7. Sears, S. M. S., and Hewett, S. J. (2021) Influence of glutamate and GABA transport on brain excitatory/inhibitory balance, *Exp. Biol. Med. (Maywood)*, **246**, 1069-1083, doi: 10.1177/1535370221989263.
8. Kolosova, N. G., Stefanova, N. A., Korbolina, E. E., Fursova, A. Z., and Kozhevnikova, O. S. (2014) Senescence-accelerated OXYS rats: a genetic model of prema-

ture aging and age-related diseases, *Adv. Gerontol.*, **27**, 336-340, doi: 10.1134/S2079057014040146.

9. Stefanova, N. A., Muraleva, N. A., Korbolina, E. E., Kiseleva, E., Maksimova, K. Y., and Kolosova, N. G. (2015) Amyloid accumulation is a late event in sporadic Alzheimer's disease-like pathology in nontransgenic rats, *Oncotarget*, **6**, 1396-1413, doi: 10.18632/ONCOTARGET.2751.
10. Kolosova, N. G., Kozhevnikova, O. S., Muraleva, N. A., Rudnitskaya, E. A., Rumyantseva, Y. V., Stefanova, N. A., Telegina, D. V., Tyumentsev, M. A., Fursova, A. Z. (2022) SkQ1 as a tool for controlling accelerated senescence program: experiments with OXYS rats, *Biochemistry (Moscow)*, **87**, 1552-1562, doi: 10.1134/S0006297922120124.
11. Stefanova, N. A., Maksimova, K. Y., Kiseleva, E., Rudnitskaya, E. A., Muraleva, N. A., and Kolosova, N. G. (2015) Melatonin attenuates impairments of structural hippocampal neuroplasticity in OXYS rats during active progression of Alzheimer's disease-like pathology, *J. Pineal. Res.*, **59**, 163-177, doi: 10.1111/JPI.12248.
12. Telegina, D. V., Antonenko, A. K., Fursova, A. Z., and Kolosova, N. G. (2022) The glutamate/GABA System in the retina of male rats: effects of aging, neurodegeneration, and supplementation with melatonin and antioxidant SkQ1, *Biogerontology*, **23**, 571-585, doi: 10.1007/s10522-022-09983-w.
13. Stefanova, N. A., and Kolosova, N. G. (2023) The rat brain transcriptome: from infancy to aging and sporadic Alzheimer's disease-like pathology, *Int. J. Mol. Sci.*, **24**, 1462, doi: 10.3390/ijms24021462.
14. Stefanova, N. A., Maksimova, K. Y., Rudnitskaya, E. A., Muraleva, N. A., and Kolosova, N. G. (2018) Association of cerebrovascular dysfunction with the development of Alzheimer's disease-like pathology in OXYS rats, *BMC Genomics*, **19**, 51-63, doi: 10.1186/S12864-018-4480-9.
15. Magi, S., Piccirillo, S., Amoroso, S., and Lariccia, V. (2019) Excitatory amino acid transporters (EAATs): glutamate transport and beyond, *Int. J. Mol. Sci.*, **20**, 5674, doi: 10.3390/ijms20225674.
16. Pajarillo, E., Rizor, A., Lee, J., Aschner, M., Lee, E. (2019) The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: Potential targets for neurotherapeutics, *Neuropharmacology*, **161**, 1-37, doi: 10.1016/j.neuropharm.2019.03.002.
17. Snytnikova, O., Telegina, D., Savina, E., Tsentalovich, Y., and Kolosova, N. (2023) Quantitative metabolomic analysis of the rat hippocampus: effects of age and of the development of Alzheimer's disease-like pathology, *J. Alzheimer's Disease*, doi: 10.3233/JAD-230706, in press.
18. Schubert, F., Gallinat, J., Seifert, F., and Rinneberg, H. (2004) Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla, *NeuroImage*, **21**, 1762-1771, doi: 10.1016/j.neuroimage.2003.11.014.
19. Kaiser, L. G., Schuff, N., Cashdollar, N., and Weiner, M. W. (2005) Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T, *Neurobiol. Aging*, **26**, 665-672, doi: 10.1016/j.neurobiolaging.2004.07.001.
20. Chang, L., Jiang, C. S., and Ernst, T. (2009) Effects of age and sex on brain glutamate and other metabolites, *Magn. Reson. Imaging*, **27**, 142-145, doi: 10.1016/j.mri.2008.06.002.21.
21. Huang, D., Liu, D., Yin, J., Qian, T., Shrestha, S., and Ni, H. (2017) Glutamate-glutamine and GABA in brain of normal aged and patients with cognitive impairment, *Eur. Radiol.*, **27**, 2698-2705, doi: 10.1007/S00330-016-4669-8.
22. Rozycka, A., Charzynska, A., Misiewicz, Z., Maciej Stepniewski, T., Sobolewska, A., Kossut, M., and Liguz-Lecznar, M. (2019) Glutamate, GABA, and presynaptic markers involved in neurotransmission are differently affected by age in distinct mouse brain regions, *ACS Chem. Neurosci.*, **10**, 4449-4461, doi: 10.1021/acschemneuro.9b00220.
23. Segovia, G., Porras, A., Del Arco, A., and Mora, F. (2001) Glutamatergic neurotransmission in aging: a critical perspective, *Mech. Ageing Dev.*, **122**, 1-29, doi: 10.1016/S0047-6374(00)00225-6.
24. Dong, Y., and Brewer, G. J. (2019) Global metabolic shifts in age and Alzheimer's disease mouse brains pivot at NAD^+/NADH redox sites, *J. Alzheimers Dis.*, **71**, 119-140, doi: 10.3233/JAD-190408.
25. Mira, R. G., and Cerpa, W. (2020) Building a bridge between NMDAR-mediated\excitotoxicity and mitochondrial dysfunction in chronic and acute diseases, *Cell. Mol. Neurobiol.*, **41**, 1413-1430, doi: 10.1007/S10571-020-00924-0.
26. Rodríguez-Giraldo, M., González-Reyes, R. E., Ramírez-Guerrero, S., Bonilla-Trilleras, C. E., Guardo-Maya, S., and Nava-Mesa, M. O. (2022) Astrocytes as a therapeutic target in Alzheimer's disease-comprehensive review and recent developments, *Int. J. Mol. Sci.*, **23**, 13630, doi: 10.3390/ijms232113630.
27. Yeung, J. H. Y., Palpagama, T. H., Wood, O. W. G., Turner, C., Waldvogel, H. J., Faull, R. L. M., and Kwakowsky, A. (2021) EAAT2 expression in the hippocampus, subiculum, entorhinal cortex and superior temporal gyrus in Alzheimer's disease, *Front. Cell. Neurosci.*, **15**, 702824, doi: 10.3389/fncel.2021.702824.
28. Babaei, P. (2021) NMDA and AMPA receptors dysregulation in Alzheimer's disease, *Eur. J. Pharmacol.*, **908**, 174310, doi: 10.1016/j.ejphar.2021.174310.
29. Kumar, A. (2015) NMDA receptor function during senescence: implication on cognitive performance, *Front. Neurosci.*, **9**, 473, doi: 10.3389/fnins.2015.00473.
30. Kumar, A., and Foster, T. C. (2018) Alteration in NMDA receptor mediated glutamatergic neurotransmission in the hippocampus during senescence, *Neurochem. Res.*, **44**, 38-48, doi: 10.1007/S11064-018-2634-4.

31. Avila, J., Llorens-Martín, M., Pallas-Bazarra, N., Bolos, M., Perea, J. R., Rodríguez-Matellan, A., and Hernandez, F. (2017) Cognitive decline in neuronal aging and Alzheimer's disease: role of NMDA receptors and associated proteins, *Front. Neurosci.*, **11**, 626, doi: 10.3389/fnins.2017.00626.
32. Cercato, M. C., Vázquez, C. A., Kornisiuk, E., Aguirre, A. I., Colettis, N., Snitcofsky, M., Jerusalinsky, D. A., and Baez, M. V. (2017) GluN1 and GluN2A NMDA receptor subunits increase in the hippocampus during memory consolidation in the rat, *Front. Behav. Neurosci.*, **10**, 242, doi: 10.3389/fnbeh.2016.00242.
33. Ge, Y., and Wang, Y. T. (2023) GluN2B-containing NMDARs in the mammalian brain: pharmacology, physiology, and pathology, *Front. Mol. Neurosci.*, **16**, 1190324, doi: 10.3389/fnmol.2023.1190324.
34. Yeung, J. H. Y., Walby, J. L., Palpagama, T. H., Turner, C., Waldvogel, H. J., Faull, R. L. M., and Kwakowsky, A. (2021) Glutamatergic receptor expression changes in the Alzheimer's disease hippocampus and entorhinal cortex, *Brain Pathol.*, **31**, e13005, doi: 10.1111/BPA.13005.
35. Qu, W., Yuan, B., Liu, J., Liu, Q., Zhang, X., Cui, R., Yang, W., and Li, B. (2021) Emerging role of AMPA receptor subunit GluA1 in synaptic plasticity: implications for Alzheimer's disease, *Cell Prolif.*, **54**, e12959, doi: 10.1111/cpr.12959.
36. Li, Y., Sun, H., Chen, Z., Xu, H., Bu, G., and Zheng, H. (2016) Implications of GABAergic neurotransmission in Alzheimer's disease, *Front. Aging Neurosci.*, **8**, 31, doi: 10.3389/fnagi.2016.00031.
37. Bi, D., Wen, L., Wu, Z., and Shen, Y. (2020) GABAergic dysfunction in excitatory and inhibitory (E/I) imbalance drives the pathogenesis of Alzheimer's disease, *Alzheimers Dement.*, **16**, 1312-1329, doi: 10.1002/alz.12088.
38. Lee, S. E., Lee, Y., and Lee, G. H. (2019) The regulation of glutamic acid decarboxylases in GABA neurotransmission in the brain, *Arch. Pharm. Res.*, **42**, 1031-1039, doi: 10.1007/s12272-019-01196-z.
39. Chattopadhyaya, B., Di Cristo, G., Wu, C. Z., Knott, G., Kuhlman, S., Fu, Y., Palmiter, R. D., and Huang, Z. J. (2007) GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual cortex, *Neuron*, **54**, 889-903, doi: 10.1016/j.neuron.2007.05.015.
40. Lau, C. G., and Murthy, V. N. (2012) Activity-dependent regulation of inhibition via GAD67, *J. Neurosci.*, **32**, 8521-8531, doi: 10.1523/JNEUROSCI.1245-12.2012.
41. Sandhu, K. V., Lang, D., Müller, B., Nullmeier, S., Yanagawa, Y., Schwegler, H., and Stork, O. (2014) Glutamic acid decarboxylase 67 haplodeficiency impairs social behavior in mice, *Genes Brain Behav.*, **13**, 439-450, doi: 10.1111/GBB.12131.
42. Kash, S. F., Johnson, R. S., Tecott, L. H., Noebels, J. L., Mayfield, R. D., Hanahan, D., and Baekkeskov, S. (1997) Epilepsy in mice deficient in the 65-KDa isoform of glutamic acid decarboxylase, *Proc. Natl. Acad. Sci. USA*, **94**, 14060-14065, doi: 10.1073/PNAS.94.25.14060.
43. Toritsuka, M., Yoshino, H., Makinodan, M., Ikawa, D., Kimoto, S., Yamamuro, K., Okamura, K., Akamatsu, W., Okada, Y., Matsumoto, T., Hashimoto, K., Ogawa, Y., Saito, Y., Watanabe, K., Aoki, C., Takada, R., Fukami, S. I., Hamano-Iwasa, K., Okano, H., and Kishimoto, T. (2021) Developmental dysregulation of excitatory-to-inhibitory GABA-polarity switch may underlie schizophrenia pathology: a monozygotic-twin discordant case analysis in human IPS cell-derived neurons, *Neurochem. Int.*, **150**, e105179, doi: 10.1016/J.NEUINT.2021.105179.
44. Benes, F. M., Lim, B., Matzilevich, D., Walsh, J. P., Subburaju, S., and Minns, M. (2007) Regulation of the GABA cell phenotype in hippocampus of schizophrenics and bipolars, *Proc. Natl. Acad. Sci. USA*, **104**, 10164-10169, doi: 10.1073/pnas.0703806104.
45. Lanoue, A. C., Dumitriu, A., Myers, R. H., Soghomonian, J. J. (2010) Decreased glutamic acid decarboxylase MRNA expression in prefrontal cortex in Parkinson's disease, *Exp. Neurol.*, **226**, 207-217, doi: 10.1016/j.expneurol.2010.09.001.
46. Wang, Y., Wu, Z., Bai, Y. T., Wu, G. Y., and Chen, G. (2017) Gad67 haploinsufficiency reduces amyloid pathology and rescues olfactory memory deficits in a mouse model of Alzheimer's disease, *Mol. Neurodegener.*, **12**, 73, doi: 10.1186/s13024-017-0213-9.
47. Ethiraj, J., Palpagama, T. H., Turner, C., van der Werf, B., Waldvogel, H. J., Faull, R. L. M., and Kwakowsky, A. (2021) The effect of age and sex on the expression of GABA signaling components in the human hippocampus and entorhinal cortex, *Sci. Rep.*, **11**, 21470, doi: 10.1038/s41598-021-00792-8.
48. Krantic, S., Isorce, N., Mechawar, N., Davoli, M. A., Vignault, E., Albuquerque, M., Chabot, J. G., Moyse, E., Chauvin, J. P., Aubert, I., McLaurin, J., and Quirion, R. (2012) Hippocampal GABAergic neurons are susceptible to amyloid-β Toxicity *in vitro* and are decreased in number in the Alzheimer's disease TgCRND8 mouse model, *J. Alzheimers Dis.*, **29**, 293-308, doi: 10.3233/JAD-2011-110830.
49. Ulrich, D. (2015) Amyloid-β impairs synaptic inhibition via GABAA receptor endocytosis, *J. Neurosci.*, **35**, 9205-9210, doi: 10.1523/JNEUROSCI.0950-15.2015.
50. Palpagama, T. H., Sagniez, M., Kim, S., Waldvogel, H. J., Faull, R. L., and Kwakowsky, A. (2019) GABAA receptors are well preserved in the hippocampus of aged mice, *eNeuro*, **6**, 1-13, doi: 10.1523/ENEURO.0496-18.2019.
51. Rissman, R. A., and Mobley, W. C. (2011) Implications for treatment: GABAA receptors in aging, down syndrome and Alzheimer's disease, *J. Neurochem.*, **117**, 613-622, doi: 10.1111/J.1471-4159.2011.07237.X.
52. Stefanova, N. A., Kozhevnikova, O. S., Vitovtov, A. O., Maksimova, K. Y., Logvinov, S. V., Rudnitskaya, E. A.,

Korbolina, E. E., Muraleva, N. A., and Kolosova, N. G. (2014) Senescence-accelerated OXYS rats: a model of age-related cognitive decline with relevance to abnormalities in Alzheimer disease, *Cell Cycle*, **13**, 898-909, doi: 10.4161/CC.28255.

53. Neff, R. A., Wang, M., Vatansever, S., Guo, L., Ming, C., Wang, Q., Wang, E., Horgusluoglu-Moloch, E., Song, W. M., Li, A., Castranio, E. L., Tcw, J., Ho, L., Goate, A., Fossati, V., Noggle, S., Gandy, S., Ehrlich, M. E., Katsel, P., Schadt, E., Cai, D., Brennand, K. J., Haroutunian, V., and Zhang, B. (2021) Molecular subtyping of Alzheimer's disease using RNA sequencing data reveals novel mechanisms and targets, *Sci. Adv.*, **7**, eabb5398, doi: 10.1126/sciadv.abb5398.