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Expression of Transporter Genes in Astrocytes for Different Neuromediators in Different Brain Departments in Rats

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Abstract

An experimental study has been carried out aiming at studying the expression of solute carriers (SLC) superfamily of the following neuromediators: glutamate, aspartate, lactate and choline. The study has been conducted on the pure culture of astroglial cells isolated from the brain in 3-day and 11-day rats. Expression of transporter genes in astrocytes for different neuromediators studied in different rat brain regions: cortex, brainstem, hippocampus.

Results of the analysis of high-performance sequencing data are presented, which show that the expression of SLC transporters of neurometransmiters (glutamate, aspartate, lactate and choline) in different parts of the rat brain increases with the development of the organism, and this process occurs unevenly. An expression of glutamate and aspartate transporters in the brainstem of 3-day rats is higher than those in the other regions. An opposite effect is observed in rats at the age of eleven days. As they get older the expression of lactate transporters becomes identical to the data from the cortex.

The results obtained can be related to the evolution of metabolism in the analyzed brain structures, as well as to the features of neuro-astrocytic bonds and participation of astrocytes in signal transduction.

Results of the study using genetic methods for the registration of neuromediators transporters make it possible to recommend the use of these methods to control the correction of different neurological disorders, including neurogenesis, neurodegenerative diseases and stroke.

Keywords: neuroglia, astrocytes, neuromediators, neurotransmitters, glutamate, aspartate, choline, lactate, gene, transporters, genetic methods.

INTRODUCTION

The urgency of work is caused by progressive incidence and disability in neurodegenerative diseases, cerebral stroke and other central nervous system (CNS) pathologies [1-5]. In this regard, the necessity for a thorough study of the mechanisms of the development of brain diseases, the pathogenesis of which is always associated with neurotransmitter imbalance, is obvious.

It is known that neuroglia cells play an important role in the functioning of the brain and nervous system as a whole. They perform a number of functions that ensure normal development, growth and work of neurons. In addition, an important role of neuroglia cells in the development of some serious diseases of the CNS has been established [6-8]. For these reasons, neuroglia has become the subject of active studies in recent years. One of the key directions is to study the connection between neurons and astrocytes, which is based on the fact that in response to neurotransmitters released by neurons, intracellular calcium concentration of astrocytes increases. Important functions of astroglia are the absorption and release of neurotransmitters; regulation of synaptic transmission and excitability of neurons [9-11]; providing nutrients, energy substrates and mediators of precursors; neurotropism; metabolism and detoxification of ammonia, drugs and hormones; waste disposal of the other cells; absorption of metals; development and support of blood-brain barrier [12]; regulation of neuronal migration during the development; and immune/inflammatory functions.

An important role is played by the ability of astrocytes to absorb the excitatory neurotransmitter - glutamate [13, 14]. This not only affects glutamatergic neurotransmission and detoxification of ammonia, but also is necessary to prevent excitotoxic trauma. The absorption of glutamate by astrocytes is caused by the work of powerful transporters. Three glutamate transporters were identified: GLT-1, GLAST, EAAT1. Disruption of glutamate transportation contributes to the development of certain neurological disorders.

Thus, astrocytes perform all the known functions of homeostasis and CNS purification: they provide structural support and determine brain structure; they establish connection between brain parenchyma and the vascular system; they play an important role in neurogenesis and the development of the nervous system. At the same time, more and more data appear that indicate participation of astroglia in the transmission of the signal and the effect on synaptic plasticity through the release of neurotransmitters, which determines the need to study an expression profile of the transporters participating in the transport of the transporters.

Transporters are integral membrane proteins that allow the movement of chemicals into and out of the cell through active and passive mechanisms. They can be classified as efflux or absorption proteins, depending on the transportation direction. The level of expression of genes encoding transport proteins can have a huge impact on the bioavailability and pharmacokinetics of various drugs. In addition, genetic variations, such as single nucleotide polymorphisms (SNP) of transport proteins, can cause differences in the absorption or efflux of drugs [15-17].

There are two superfamilies of transport proteins that have a significant effect on absorption, distribution and excretion of drugs. These are representatives of ATP-binding cluster superfamily (ABC) and **SLC** superfamily. SLC genes encode membrane transporters. At the present time, we know 55 families among genes of the human SLC superfamily with 362 supposedly functionally protein-coding genes [18, 19]. Members of SLC superfamily include membrane channels, facilitating transporters and secondary active transporters. Examples of some of the endogenous solutions transported by these proteins are steroid, thyroid hormones, leukotrienes and prostaglandins. In addition, SLC transporters are important participants in the transportation of a large number of drugs.

Due to this, systemic studies of astroglia functioning in various parts of the brain, its influence and connection with neurons help to better understand the role of this population of cells in the CNS, while the idea of a difference in the number of carriers of neurotransmitters can contribute to the creation of highly effective drugs.

Therefore, the **aim of** this study was to study an expression of SLC transporters of different neurotransmitters in pure culture of astroglial cells isolated from different regions of the brain (cortex, hippocampus and brainstem) in 3-days and 11-days rats.

In this paper, we made an emphasis on the study of the expression of transporters of some of the most important neurotransmitter/signaling systems. Glutamic acid (glutamate) is the most common exciting neurotransmitter in the vertebrate nervous system, in the neurons of cerebellum and spinal cord. Its excess is fraught with the development of excitotoxic damage to neurons. Aspartic acid (aspartate) is an exciting neurotransmitter in the neurons of the cerebral cortex. Acetylcholine performs neuromuscular transmission. Also, it is the main neurotransmitter in the parasympathetic nervous system, the only derivative of choline among neurotransmitters. Lactate is a marker of energy supply and energy deficit in brain cells. Investigated molecules and their transporters are presented in Table 1.

MATERIALS AND METHODS

The studies were performed on 24 *Rattus norvegicus* rats of both sexes at the age of 3 (12 rats) and 11 days (12 rats).

During the work, we optimized the protocol of immunomagnetic separation in order to isolate astrocytes from different parts of the brain. High-performance sequencing of material obtained from a pure cell suspension was performed. We used the method of immunomagnetic separation in order to obtain pure culture of astrocytes.

Expression of SLC genes was analyzed by the final transcript assemblies following Cuffmerge procedure, and then it was measured in fpkm. Not only data obtained from different regions of the rat brain (cortex, brainstem, hippocampus) were compared, but also gene expression data for the specimens of different ages (3 days and 11 days old). The experiment was carried out twice for 3-day and twice for 11-day rats.

Immunomagnetic separation was performed by using the Anti-GLAST (ACSA-1) MicroBead Kit (Miltenyi Biotec) in accordance with the manufacturer's recommendations. This technology is based on the use of super-magnetic microparticles conjugated with highly specific monoclonal antibodies. ACSA-1 is an antibody specific for the extracellular epitope of transmembrane glycoprotein of astrocytes (GLAST). GLAST is Na+ dependent L-glutamate transporter that plays an important role in removing L-glutamate neurotransmitters from extracellular space and maintaining normal physiological state of the cells. This protein is also expressed predominantly on the surface of astrocytes in mammals at the early stages of the development.

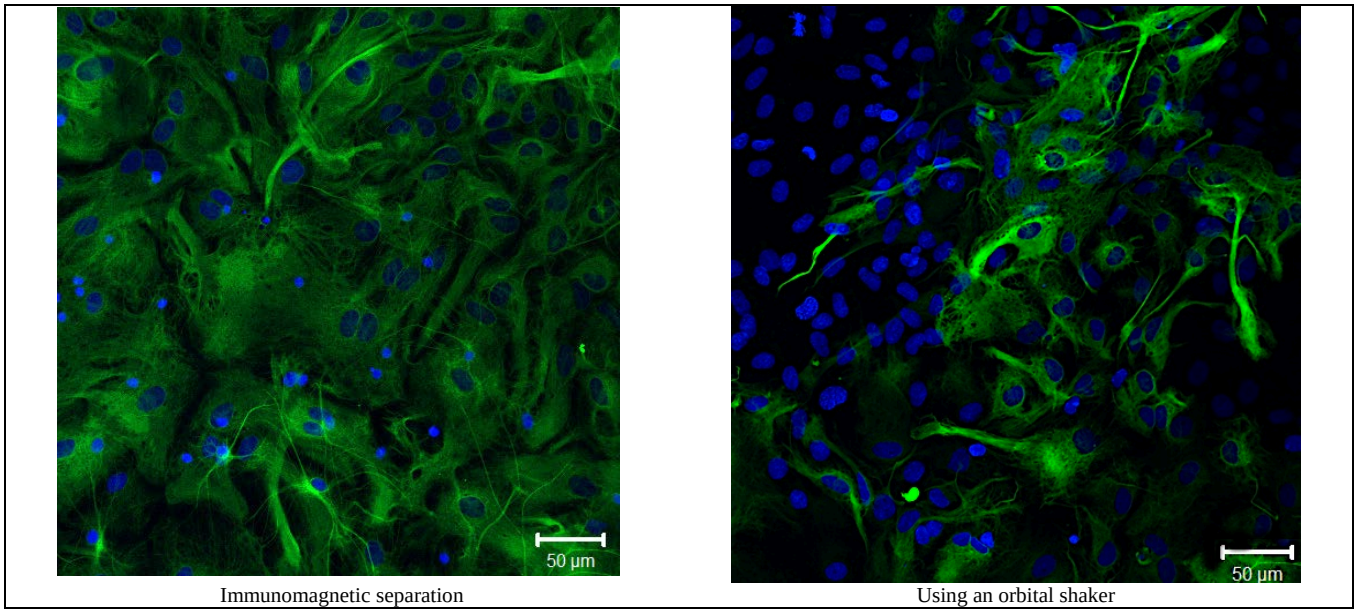
Evaluation of the purity of the experiment was carried out on the prepared fixed preparations with a confocal Carl Zeiss LSM 780 microscope. Samples were taken from astrocyte cultures that underwent immunomagnetic separation methods and using an orbital shaker. Then cell nuclei were stained by using the fluorescent dye DAPI (4', 6-diamidino-2-phenylindole). Also, preparation was stained for GFAP (glial acidic fibrillar protein), which is specific intermediate filament of astrocytes (Fig. 1)

On the images obtained, we can see astrocyte cells stained in green, with bright blue nuclei. When comparing the luminescence level of DAPI and GFAB dye, it was found that other cells were present in the sample of cells isolated by immunomagnetic separation. However, their number does not exceed 4% of the total cell volume, which is permissible.

Isolation of mRNA. RNA was isolated from astrocytes of three brain regions (cortex, brainstem, hippocampus) by using the RNeasy Mini kit ("Qiagen", Germany) according to the instructions. Previously, the cells were homogenized with Minilys homogenizer ("Berlin Technologies", France). The quality of the isolated RNA was verified by gel electrophoresis in an agarose gel. Electrophoresis was performed in 1% agarose gel prepared on the basis of TAE buffer (Tris-Acetat-EDTA) at the concentration of 50x with the addition of intercalating dye-ethidium bromide. TAE was used as an electrode buffer. The sample was applied to a gel with an appropriate buffer (0.25% bromophenol blue, 0.25% xylencyanol, EDTA (pH 8.0) 10 mM, glycerol 50%). GeneRuler Express DNA Ladder ("Fermentas/Thermo Scientific", USA) was used as a marker. Conditions for electrophoresis - voltage, current or power, and time - were set based on the dimensions of the chamber and the size of the sample applied to the gel. At the end of the electrophoresis, the gel was gently moved into the transilluminators (VersaDoc and GelDoc, Bio-Rad). Concentration of total isolated RNA was measured by using the Qubit 2.0 fluorometer ("Invitrogen", USA) and Qubit RNA BR Assay Kit reagent kit ("Invitrogen", USA). From RNA obtained, we isolated mRNA by using the commercial kit NEBNext Poly (A) mRNA Magnetic Isolation Module (New England BioLabs, USA).

Table 1. Signal molecules under the study and their transporters

Signal molecule	Description	SLC transporters*
Glutamate and aspartate	Glutamate and aspartate are the most common and studied neurotransmitters. They are involved in the learning process and the formation of long-term memory by interacting with NMDA receptors, which in turn participate in synaptic plasticity. Glutamate plays a role in conducting a nerve impulse, volumetric neurotransmission, and also plays an important role in synaptogenesis and regulation of growth cones during the development of the brain. Aspartate is used as an amine group donor to synthesize glutamate and glutamine in astrocytes.	SLC1a1 (EAAT3), SLC1a2 (EAAT2), SLC1a3 (EAAT1), SLC1a4 (ASCT1), SLC1a5 (AAAT), SLC7a11 (CCBR1)
Glutamate		SLC17ab (VGLUT2) SLC17a7 (VGLUT1) SLC25a18 (GC2), SLC2a1 (GLUT1)
Lactate	Lactate is an important source of energy that enters the neurons through astrocytes. Lactate is transported through monocarboxylate transporters (MST), proton-crosslinked membrane carriers that transport monocarboxylates through the cell membrane, including lactate and pyruvate. Lactate is a moderator of one of the types of glutamate receptors and enhances their activity. Its transportation plays an important role in the formation of long-term memory. Until recently, these functions were considered as the main ones, but a group of British scientists obtained data that allowed us to speak of lactate as about new signaling molecule (22, 29).	SLC16a1 (MCT1), SLC16a3 (MCT4), SLC16a7 (MCT2)
Choline / acetylcholine	Acetylcholine is one of the most important neurotransmitter of the CNS. It takes part in the transfer of impulses from different parts of the brain. At high concentrations it has an inhibitory effect on synaptic transmission. Violation of acetylcholine metabolism leads to unfavorable changes in brain function. In Alzheimer's disease, the level of this mediator is reduced, which leads to weakening of memory. Acetylcholine is synthesized by the enzyme acetylcholinesterase from choline and activated acetic acid (acetylcoenzyme A).	SLC5a7 (CHT), SLC44a1 (CTL1), SLC44a2 (CLT2), SLC44a5 (CLT5).
* Expressing in the brain cells		



Immunomagnetic separation Using an orbital shaker

Fig. 1. Combined image of astrocytes of the rat brainstem isolated with immunomagnetic separation (on the left side) and the method by using an orbital shaker (on the right side), and stained cell nuclei. Green staining - cells expressing GFAP (stained by using secondary antibodies), blue staining - cell nuclei (stained by using DAPI)

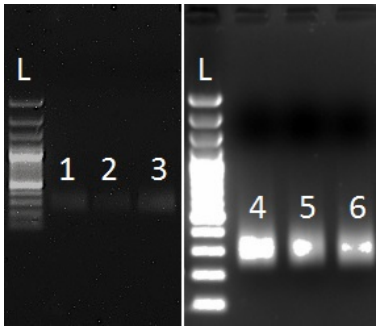


Fig. 2. Electrophoregram of mRNA libraries: 1 - mRNA of hippocampus of 3-day rats; 2 - mRNA of the brainstem of 3-day rats; 3 - mRNA of the cerebral cortex of 3-day rats; 4 - hippocampal mRNA of 11-day rats; 5 - mRNA of the brainstem of 11-day rats; 6 - mRNA of the cerebral cortex of 11-day rats; L - (Ladder) marker of DNA lengths (100-3000 kb)

Preparation of mRNA libraries. mRNA libraries of astrocytes of the cortex, brainstem, and hippocampus of rat brain were prepared by using the NEBNext mRNA Library Prep Master Mix Set for Illumina kit (New England BioLabs, USA) in accordance with the instructions attached. Purification of fragmented mRNA was performed by using the RNeasy Mini kit (Qiagen, Germany) in accordance with the enclosed RNA CleanUP protocol. Selection of fragments of the DNA library along the sequence length was carried out by using the Agencourt AMPure XP Beads resuspended magnetic particles (Beckman Coulter, Inc.) in accordance with kit recommendations NEBNext mRNA Library Prep Master Mix Set for Illumina. Concentration of the libraries was measured by using the Qubit 2.0 fluorometer and the Qubit dsDNA HS Assay Kit (Invitrogen, USA). The lengths of fragments of the finished library were checked by gel electrophoresis in the agarose gel (Fig. 2).

Sequencing and data analysis. Studies of mRNA profiles in some parts of the brain were performed by using high-throughput sequencing on MiSeq device (Illumina, USA) by using MiSeq Reagent Kit v2 (300 cycles), MiSeq Reagent Kit v2 (500 cycles), MiSeq Reagent Kit v3 (150 cycles) and PhiX Control Kit v3 (Illumina, USA). After the sequencing, pairwise readings for each sample were obtained. Raw files were automatically uploaded to the cloud server Illumina BaseSpace (https://basespace.illumina.com), where the primary processing and the process of converting the fluorescence intensity signal to FastQ nucleotide sequence format were made (Fig. 3).

The quality of the data was analyzed by using FastQC software [20] (Galaxy Version 0.69), the removal of adapters and nucleotides with unsatisfactory quality was carried out by using Trimmomatic software [21] (Galaxy Version 0.36.3) with the following parameters SLIDINGWINDOW:4:20 MINLEN:53 (Fig. 4).

The readings thus prepared were subjected to further analysis using the respective software: mapping of pair readings to a known genome was carried out with the help of TopHat (Galaxy Version 2.1.0) [22, 23]; determination of differentially expressed genes – by using DESeq [24] (version 1.28.0). The scheme of this analysis is shown in Fig. 5.

Expression of SLC transporter genes was analyzed according to the final transcript assemblies after processing in DESeq software and measured in normalized read counts. We have compared not only the data obtained from different regions of the rat brain (cortex, brainstem, hippocampus), but also the data on gene expression in individuals of different ages (3 days and 11 days).

The statistical significance of the differences obtained in the expression levels of genes was determined by taking into account the false discovery rate (FDR) -Benjamini-Hochberg. Analytical statistics were assessed by using parametric and nonparametric criteria. Differences were considered significant at $p < 0.05$.

RESULTS

It has been established that the level of expression of glutamate and aspartate SLC transporters in 3-day rats in cells of the cerebral cortex is significantly lower than that in the brainstem and hippocampal cells. In 11-day rats, the expression in the cells of the hippocampus and cortex increases and is identical on the average. The indices in the brainstem cells increase, but they are lower than those in the other regions (Fig. 6). Uneven distribution of transporters of the main excitatory amino acids of the CNS in different parts of the brain is caused by their evolutionarily developed tasks.

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Fig. 3. Fragment of the file in FASTQ format

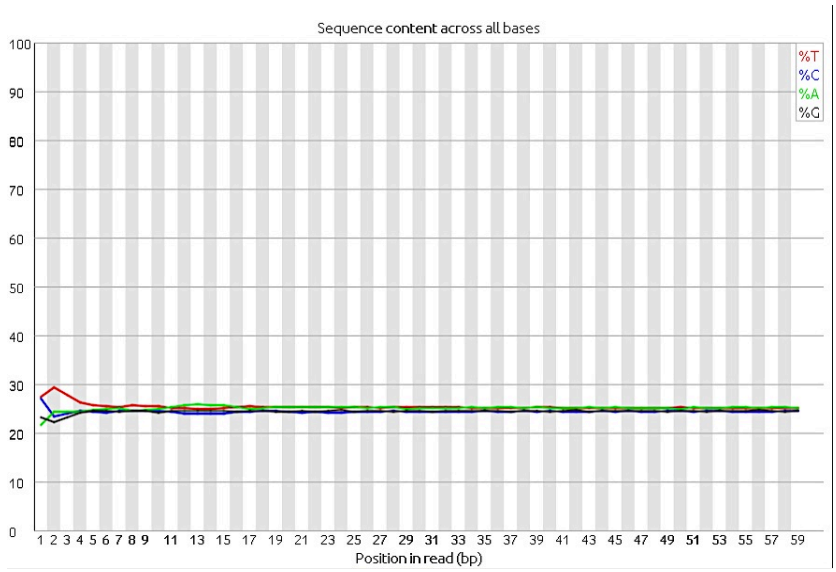

Fig. 4. Analysis of the distribution of quality parameters of read nucleotides with FastQC program. The ordinate represents the frequency of each nucleotide at the given position, along the abscissa axis. Position in read is the position of the nucleotide in reading

Analysis of the results of studying the expression of glutamate SLC transporters in astrocytes of the hippocampus, brainstem and cerebral cortex of rats of different ages showed an increase in the expression of almost all studied glutamate transporters as the rats grew in all parts of the brain. The most pronounced differences were found at the expression level of SLC17a7 transporter gene, which was 9.7 times higher in the cortex of 3-day rats, 8 times higher in the brainstem, 10.5 times higher in the hippocampus than those in 11-day rats ($p < 0.05$). On the one hand, this may indicate an increase in the exciting activity of the brain. However, on the other hand, in terms of body weight, most differences in transporter expression are compensated. In addition, an expression of SLC17ab glutamate transporter of astrocytes, on the contrary, is higher in 3-day rats than that in 11-day rats, although these changes are not statistically reliable. Consequently, the main task of glutamate transporters located on the membranes of neuroglia is the rapid removal of excess glutamate from the extracellular space (Table 2). This is a protective mechanism for physiological control of excitotoxicity. In case of various brain damage (ischemic, neurodegenerative) glutamate transporters can work in the opposite direction, so that glutamate can be accumulated outside the cell. This process results in large amounts of calcium ions entering the cell through NMDA receptor channels, which in turn causes damage and even cell death as a result of excitotoxic damage.

Thus, an expression of SLC transporters transporting glutamate in astrocytes corresponds to the physiological level of the request and is in balance between protection against excitotoxicity, on the one hand, and the level of excitatory activity, on the other hand.

This conclusion is also confirmed by the fact that the maximum expression level of almost all studied glutamate transporters of astrocytes was found in the brainstem ($p < 0.05$). This is caused by physiological need to maintain a balance between the processes of excitation and inhibition, especially in the reticular formation of the brainstem.

Analysis of the results studying the expression of transporter-responsive acetylcholine SLC transporters in the astrocytes of hippocampus, brainstem and cerebral cortex also showed an increase in the expression of almost all the studied transporters as the rats grew in proportion to the gaining of their body weight. The differences were most evident in SLC44a1 transporter, which was 3.6-fold higher in the cortex of 3-day rats, 3.15 times higher in the brainstem, and 2.7 times higher in

the hippocampus than those in 11-day rats ($p < 0.05$). The only exception was SLC5a7, which was expressed in the cortex of 3-day rats on the average 3.2 times higher than in 11-day rats. It was found that the highest levels of expression of SLC choline transporters in 3-day rats were observed in the brainstem, and the lowest levels - in the cortex. In 11-day specimens, the expression level in the cortex and hippocampus was almost identical, it was somewhat lower than that in the brainstem (Table 3).

Acetylcholine plays an important role as the CNS mediator. It is the main neurotransmitter of neuromuscular transmission, without which the movement is impossible. Acetylcholine is involved in the transmission of impulses in different parts of the brain. Its small concentrations facilitate, while large concentrations inhibit synaptic transmission. In addition, acetylcholine is an important neurotransmitter of parasympathetic nervous system. This explains different contents of acetylcholine transporters in different parts of the rat brain.

Expression of SLC-transporters of lactate in 3-day rats in the brainstem and hippocampus cells of the brain was higher than in cortical cells. In 11-day specimens, expression in the cells of the cortex and brainstem increased, and differed significantly from that in the hippocampus (Fig. 7). Age differences of the lactate transporters studied by us testify to an increasing energy demand of the cerebral cortex as mammals grow up, which correlates with the speed of their training and development. The absence of statistically significant differences in this indicator in the hippocampus indicates the stationary needs for energy supply and, accordingly, the total transmitter activity in the most evolutionarily ancient brain department, in the hippocampus, responsible for the transition of short-term memory to long-term in both 3-day and 11-day rats.

Generalized results of the experimental genetic study are presented on the heat map (Fig. 8).

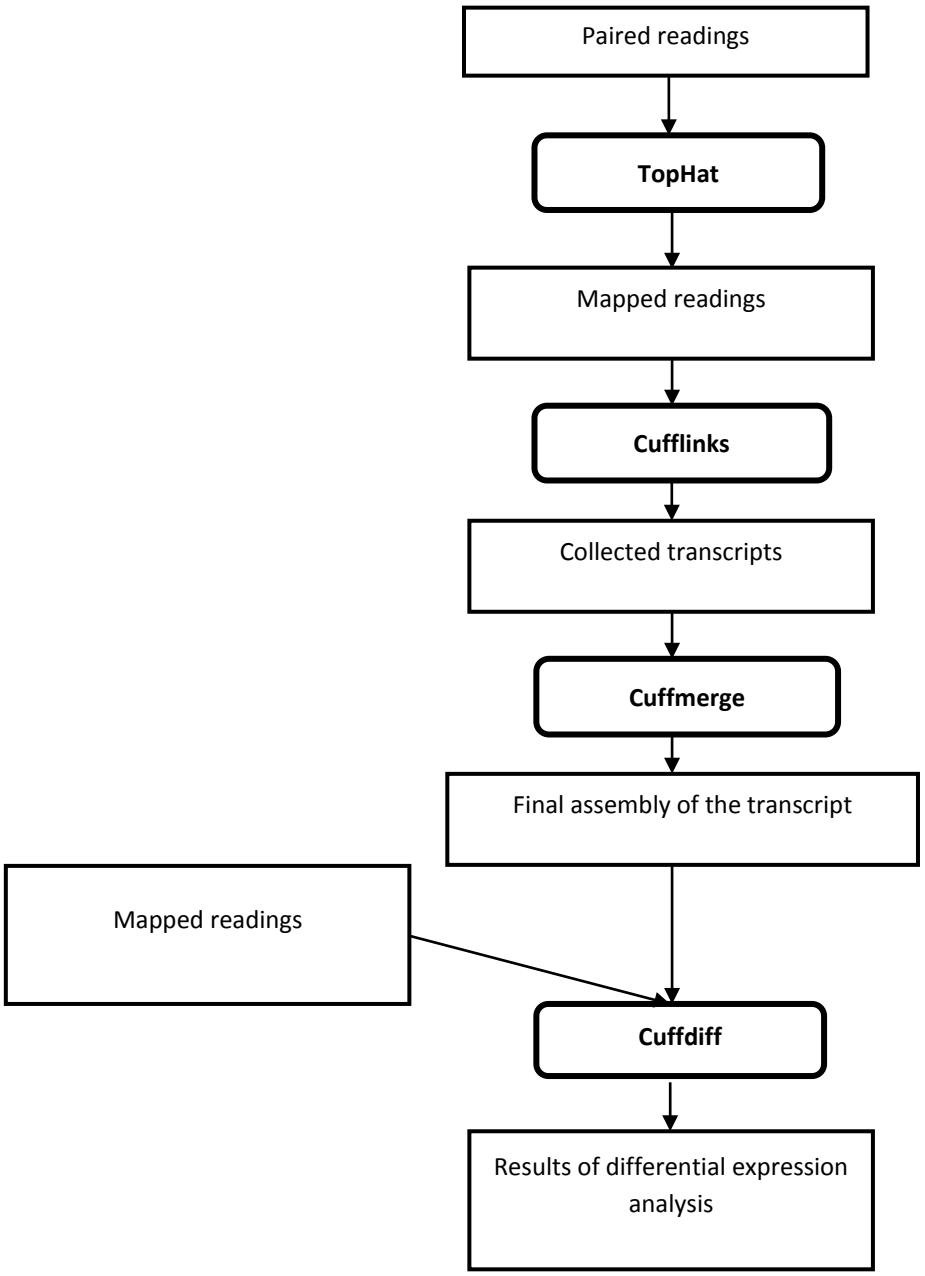

Fig. 5. Algorithm for RNA-Seq analysis for obtaining astrocyte expression profile

Table 2. Expression of SLC transporters responsible for glutamate transfer in astrocytes of the cortex, brainstem and hippocampus in rats

	SLC17ab (VGLUT2)		SLC17a7 (VGLUT1)		SLC25a18 (GC2)		SLC2a1 (GLUT1)	
Age of rats	3-day	11-day	3-day	11-day	3-day	11-day	3-day	11-day
Cortex	2.6±0.3	1.3±0.2	19.1±2.1	187±11*	63±6	164±10*	12.7±1.8	39±4*
Brainstem	7.1±0.5	4.3±0.4	0.5±0.04	4.0±0.3*	190±9	317±19*	22.3±2.1	61±7*
Hippo-campus	5.4±0.5	3.5±0.4	18±1.5	189±10*	62±5.4	210±13*	21±1.6	28±1.8
* - significant difference between groups (3-day and 11-day rats) at p <0.05								

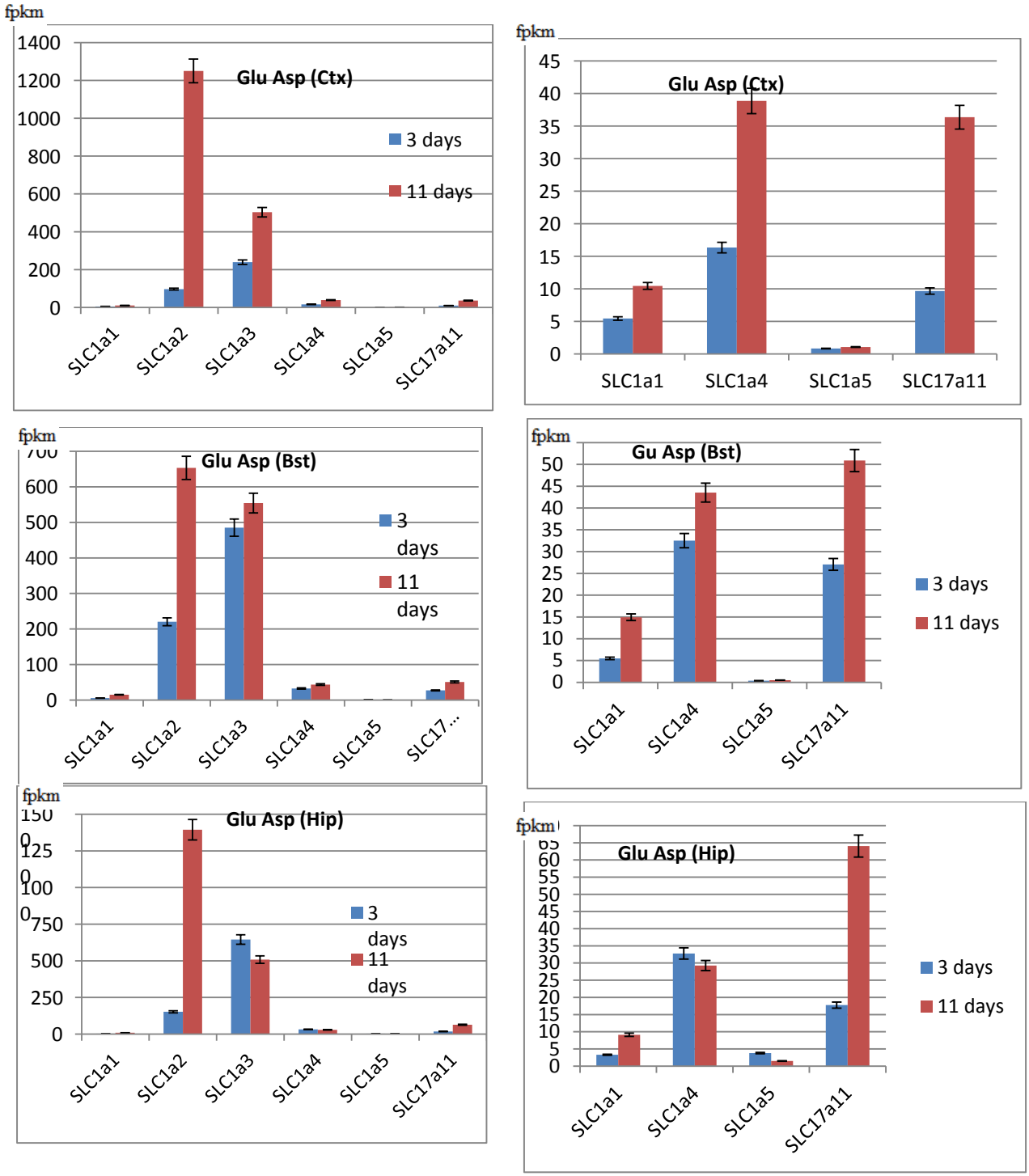

Fig. 6. Expression of SLC transporter in astrocytes of the cortex, brainstem and hippocampus, responsible for the transfer of glutamate and aspartate

Table 3. Expression of SLC transporters responsible for choline transportation in astrocytes of the cortex, brainstem and hippocampus of rats

	SLC5a7 (CHT)		SLC44a1 (CTL1)		SLC44a2 (CLT2)		SLC44a5 (CLT5)	
Age of rats	3-day	11-day	3-day	11-day	3-day	11-day	3-day	11-day
Cortex	0.16±0.02	0.05±0.01*	9.9±0.5	32.5±2.4*	12.5±0.2	21.1±2*	4.0±0.2	5.2±0.3
Brain stem	0.15±0.01	0.29±0.03*	25.1±0.2	79±7*	20.5±0.1	27±0.2	4.9±0.3	7.0±0.7
Hippo-campus	0.11±0.01	0.07±0.01	12±1.1	32.2±2.1*	21±2	21.9±2	4.1±0.4	3.3±0.3
* - significant difference between groups (3-day and 11-day rats) at p <0.05								

DISCUSSION

At the present time, the question on the functions and role of astroglia in the work of the brain is one of the most prevalent ones in neuroscience. There is no doubt that the astrocyte network is not only the “skeleton” of the neural network. It provides its nutrition, protection, and also takes part in the transmission of the exciting signal and the neurotransmitters’ metabolism. The main topics of scientific discussions are still detailed mechanisms of interaction between neurons and astrocytes, which emphasizes an importance of studying the transport of molecules.

Astrocytes are involved in all known functions of homeostasis and purification in the CNS: they provide structural support and determine brain structure; establish a connection between parenchyma of the brain and the vascular system; and play an important role in neurogenesis and the development of the nervous system. Therefore, an in-depth study of astrocyte-neuronal interconnection through the evaluation of transporter expression for the most important neurotransmitters is extremely relevant.

In this study, a suspension of astrocytes with a purity of 96-97% was obtained by the method of immunomagnetic separation. These figures were repeated when checked by staining for astrocytic markers after each isolation, which allowed to speak about the reliability of the study. We used modern methods for isolating a pure cell suspension of astrocytes, high-throughput sequencing, applied an algorithm for analyzing gene expression data for SLC transporter neurotransmitters. Preparation of homogeneous population of astrocytes was carried out by using the method of immunomagnetic separation. The purity of this method was checked, and mRNA libraries of the resulting material for sequencing were described in detail.

The analysis of high-performance sequencing data has shown that the expression of SLC-transporters of neurometransmiters (glutamate, aspartate, lactate, choline) in different parts of the brain of the rat increases unevenly as the body develops. The greatest interest is represented by the results related to the brainstem cells as the expression parameters are very different from their levels in the cortex and hippocampus, where expression increases for all neurotransmitters and is almost identical (in the case of choline, glutamate and aspartate). In the brainstem, the expression level of the choline transporter is higher than that in the other regions, both in 3-day and 11-day specimens. An expression of lactate transporters becomes identical with age to the data of the cortex. Expression of glutamate and aspartate transporters in the brainstem in 3-day rats is higher than that in the other regions. However, we can see the reverse situation in 11-day rats. It can be assumed that this is related to the metabolism in this brain structure or to the peculiarity of neuro-astrocytic connections and participation of astrocytes in signal transduction. In order to confirm any of the theories, the team of authors is currently conducting additional studies [25, 26].

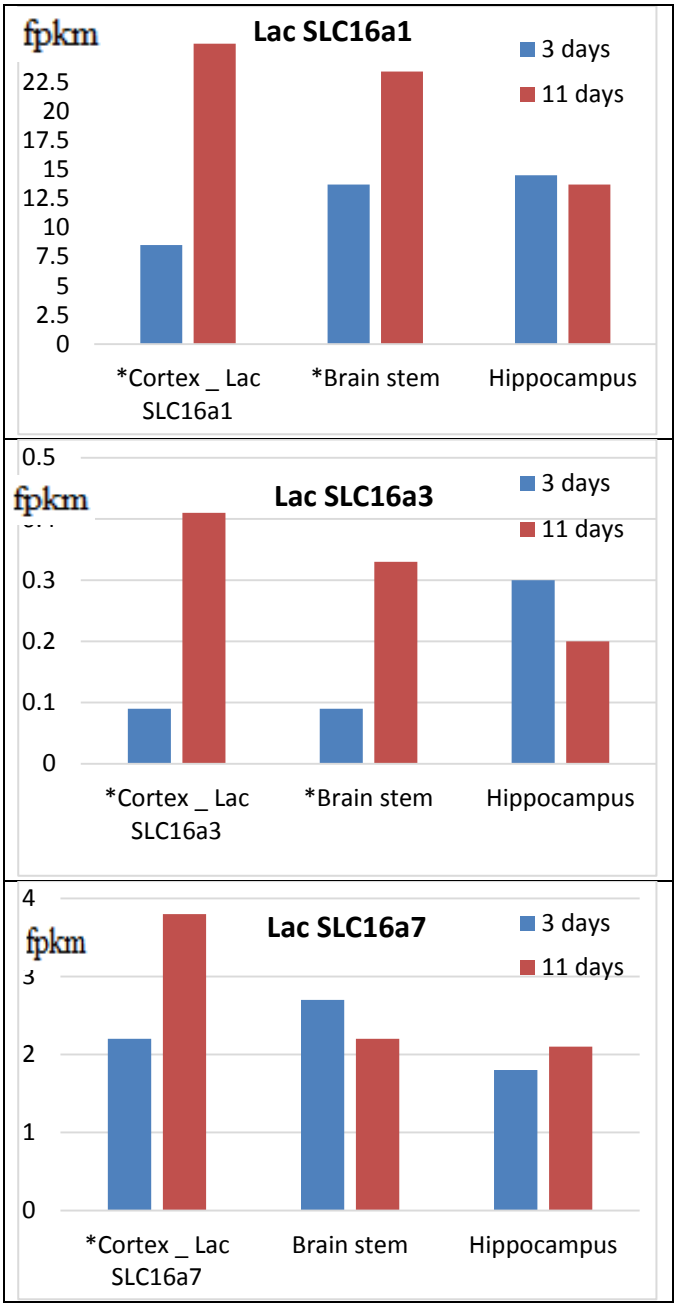

Fig. 7. Expression of SLC transporters in astrocytes of the cortex, brainstem and hippocampus of rat responsible for lactate transportation (* - significant difference between groups (3-day and 11-day rats) at p <0.05)

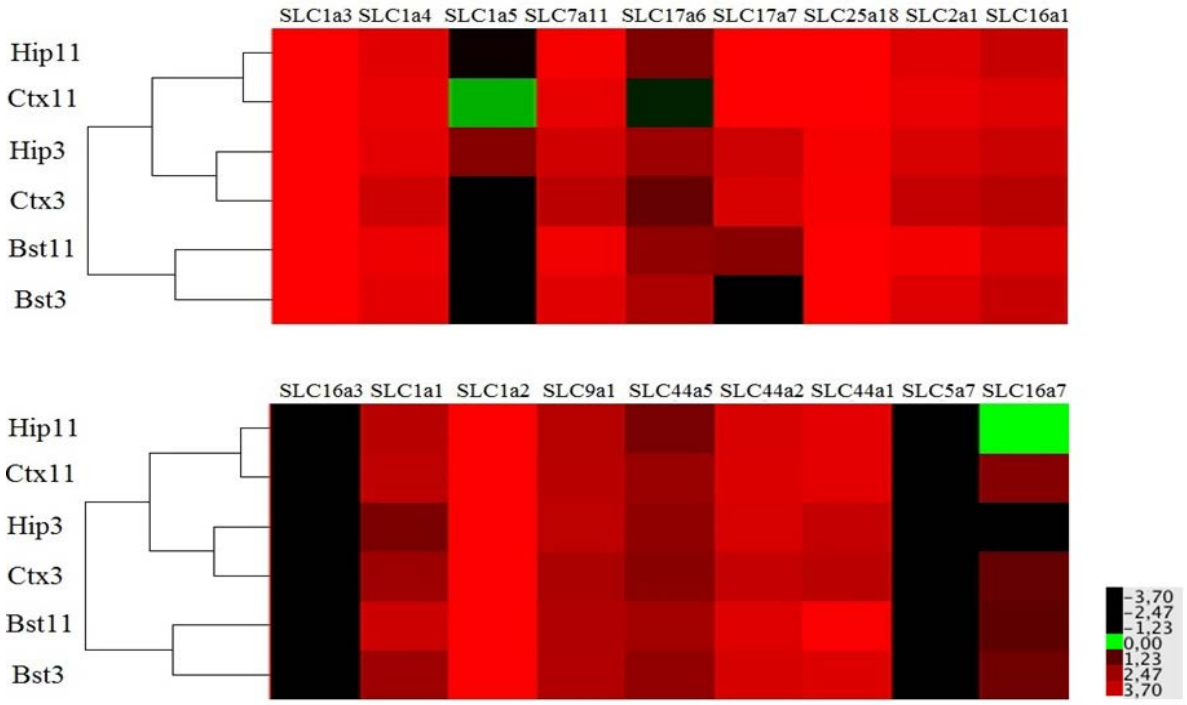

Fig. 8. Heat map of expression of SLC transporters in astrocytes of different parts of the rat brain: Ctx3 is the cerebral cortex of 3-day rats, Ctx11 is the cerebral cortex of 11-day rats, Hip3 is the hippocampus of the brain of 3-day rats, Hip11 is the hippocampus of the brain of 11-day rats, Bst3 is the brainstem 3-day rats, and Bst11 is the brainstem of 11-day rats

CONCLUSION

Results of the experimental study on the qualitative and quantitative analysis of the expression of SLC transporters of different neurotransmitters in different parts of the rat brain have shown the age and regional differences in the expression levels of the transporters of different neurotransmitters. Based on the results of this work, one can also judge about the nature of astrocyte's response to stimulation, since the number of signal molecules that can be released in response to the stimulus is directly related to the amount of transport proteins in the cell. The data obtained characterize an expression of SLC transporters of neurotransmitters in astrocytes of different parts of the brain. The findings obtained suggest that the astrocytes of several regions of the brain, namely hippocampus, cortex and brainstem, release different amounts of glutamate, aspartate, lactate and choline in response to the same stimulus. These molecules have a significant effect on the development and vital functions of the body. Failure in the work of almost any of these transporters is associated with the development of neurological diseases (Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, multiple sclerosis, stroke, etc.). The idea of the difference in the number of transporters of these neurotransmitters in the future will help to identify the foci of many diseases, and this in turn will contribute to the creation of targeted drugs.

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