

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository:<https://orca.cardiff.ac.uk/id/eprint/119561/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Davydova, T. V., Gruden, M. A., Kudrin, V. S., Narkevich, V. B., Vetrile, L. A., Zakharova, I. A. and Sewell, Robert 2019. Effect of antibodies to glutamate on age-related memory changes in C57Bl/6 mice. *Bulletin of Experimental Biology and Medicine* 166 (3) , pp. 326-329. 10.1007/s10517-019-04343-0

Publishers page: <http://dx.doi.org/10.1007/s10517-019-04343-0>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Effect of Antibodies to Glutamate on Age-Related Memory Changes in C57Bl/6 Mice

T. V. Davydova¹, M. A. Gruden², V. S. Kudrin³, V. B. Narkevich³, L. A. Vetrile¹,
I. A. Zakharova¹, and R. D. E. Sewell⁴

¹Research Institute of General Pathology and Pathophysiology; ²P. K. Anokhin Research Institute of Normal Physiology; ³V. V. Zakusov Research Institute of Pharmacology, Moscow, Russia; ⁴School of Pharmacy and Pharmaceutical Sciences, Cardiff University, Cardiff, UK.
Address for correspondence: dav-ta@yandex.ru. T. V. Davydova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 166, No. 9, pp. 296-299, September, 2018 Original article submitted May 17, 2018

Chronic intranasal administration of antibodies to glutamate to aging C57Bl/6 mice improved passive avoidance conditioning, had no effect on horizontal and vertical locomotor activity, but slowed locomotion in the open-field test. Administration of antibodies to glutamate increased the content of dopamine and its metabolites in mouse hippocampus, but had no effect on the metabolism of neurotransmitter amino acids. In the frontal cortex, antibodies to glutamate did not affect neurotransmitter metabolism, but increased the level of both excitatory and inhibitory amino acids without changing their ratio.

Key Words: memory; antibodies to glutamate; neurotransmitters; aging

In the context of steadily increasing life expectancy, the search for new drugs and approaches to prevention and treatment of age-related changes in the elderly and old people becomes one of the most important problems of modern medicine. Cognitive disturbances developing with age impair quality of life and require timely correction, for example, using neuroimmunological methods. Using experimental pathology models, we have previously demonstrated anti-amnesic properties of polyclonal monospecific antibodies to glutamate (ATGlu). Single intranasal administration of AT-Glu in an effective dose of 250 mg/kg reduced the severity of memory impairment in mature Wistar rats with experimental Alzheimer's disease modeled by administration of a neurotoxic fragment A β ₂₅₋₃₅ impairing passive avoidance learning into the Meiner's nucleus of the brain [1]. The anti-amnesic effect of AT-Glu on cognitive dysfunction was also confirmed in experiments with amyloidogenic structures of the proinflammatory protein S100A9 involved in the amyloid cascade in Alzheimer's disease, when they induced impairment of conditioned passive avoidance reflex (CPAR) in aging C57Bl/6 mice. It was shown that co-administration of fibrillar or oligomeric S100A9 forms with AT-Glu did not impair memory in aging animals

[2,3,7]. These facts seem to be indicative of anti-anamnestic effects of AT-Glu in cognitive deficit. However, the mechanisms of action of AT-Glu remain poorly understudied. The aim of this study was to evaluate the effects of AT-Glu on mnemonic functions, as well as on specific features of neurotransmitter content in the relevant brain structures (hippocampus and cortex) of C57Bl/6 mice during aging.

MATERIALS AND METHODS

Experiments were performed on 12 month-old male C57Bl/6 mice (n=28) weighing 32.2 ± 1.8 g. The animals were kept under standard vivarium conditions with free access to water and food with 12/12 h light/ dark regimen. The experiments were carried out in compliance with requirements of the European Community Council Directive 86/609/EEC. Polyclonal monospecific AT-Glu were obtained according to the previously described protocol [6]. Male Chinchilla rabbits were immunized with glutamate conjugate with BSA carrier protein using a standard scheme. The protocol for Glu-BSA conjugate preparation was described previously [11]. Purified polyclonal monospecific AT-Glu were used in experiments in a titer of $1:1024 \pm 1:16$. The animals were divided into two groups. Control mice (n=14) received saline in a volume of 4 μ l for 14 days via an intranasal route and experimental group (n=14) received purified AT-Glu dissolved in saline in a dose of 250 μ g/kg in a volume of 4 μ l for 14 days (alternately in each nostril). In 24 h after solution administration, passive avoidance was conditioned in a chamber consisting of a light ($15.5 \times 15.5 \times 19$ cm) and a dark ($9 \times 9 \times 17$ cm) compartments. The floor in each compartment was made of metal rods with a diameter of 0.3 cm and a distance between them of 0.9 cm. The compartments were connected with a hole in the mutual wall with a guillotine door. CPAR was carried out according to a standard previously described method [3]. The latency of transition to the dark compartment was recorded on experimental day 1 (LP1, sec) and 2 (LP2, sec). The observation period for each animal was 300 sec (starting from the moment when the door was opened on training and testing days). The strength of animals' memory for electric shock was determined by the difference in the latent periods of animal's transition to the dark chamber during passive avoidance training and 24 h after training on the day of testing (Δ LP, sec). On day 3 after CPAR, locomotor activity of mice was assessed in the open field using automated AutoTrack test in the Opto-Varimex system (Columbus Instruments) for 6 min; track distance (cm), time of movement and rest time (in sec) and the number of rearings were recorded. Upon completion of behavioral experiments, all animals were decapitated, the brains were removed in cold, and the hippocampus and frontal cortex samples were isolated. Samples of cerebral structures were used for estimation of the level of biogenic amines: dopamine (DA), serotonin (5-HT), norepinephrine (NE) and metabolites of DA (DOPAC, HVA, and 3-MT) and 5-HT (5-HIAA), parameters of their metabolism, as well as the concentration of neurotransmitter amino acids: aspartate, glutamate, glycine, taurine, and GABA. The level of neurotransmitters was determined by HPLC with electrochemical detection (HPLC/ED) on a LC-304T chromatograph (BAS) with a Rheodyne 7125 injector, loop volume for applying samples of 20 μ l [2.7]. The concentration of monoamines in the samples (nmol/g of tissue) was calculated by the method of "internal standard" based on the ratios of peak areas in the standard mixture and in the experimental sample. The content of neurotransmitter amino

acids was determined by HPLC/ED by the standard method [9]. The column was calibrated with a mixture of aspartic, glutamic acids, taurine, and GABA in a concentration of 0.1 $\mu\text{mol/ml}$ in 0.1 N HClO_4 . Concentration of neurotransmitter amino acids was expressed in $\mu\text{mol/g}$ of tissue. The data were statistically processed using Statistica 7.0 software, intergroup differences were analyzed using Mann-Whitney U test.

RESULTS AND DISCUSSION

Intranasal administration of AT-Glu for 15 days improved CPAR in aging C57Bl/6 mice, which manifested in a more than 2-fold increase in the memorization level: ΔLP value was 190.9 ± 21.2 sec vs. 90.5 ± 19.9 sec in controls ($p < 0.05$).

In the hippocampus, DA level decreased by 70.7% against the background of AT-Glu administration while the content of its metabolites 3-MT and DOPAC increased by 70 and 36%, respectively; HVA concentration decreased by more than 50%. Under the influence of AT-Glu, the vector of DA metabolism coefficients DOPAC/DA shifted by 40% and HVA/DA remained unchanged (Table 1). AT-Glu administration produced no significant effect on 5-HT and NE metabolism in the hippocampus (Table 1) and on DA, NE, and 5-HT content in the frontal cortex (Table 1). At the same time, the effect of chronic AT-Glu administration on the metabolism of neurotransmitter amino acids in the frontal cortex and its absence in the hippocampus (Table 2) attract attention. Experimental animals showed a generalized increase in the concentration of both excitatory and inhibitory amino acids: asparagine (by 58%), glutamate (by 100%), glycine and taurine (by 70%), GABA (by 80%) upon maintenance of normal ratio between them (Table 2). Thus, in aging C57Bl/6 mice, an improvement in passive avoidance learning was found in case of 2-week intranasal AT-Glu administration in a dose of 250 $\mu\text{g/kg}$. AT-Glu had a protective effect on mnemonic functions of animals with age-related changes. This effect seems to be associated with the influence of AT-Glu on neuronal apoptosis [4,5]. AT-Glu administration did not change horizontal or vertical motor activity in the open field, however, in the case of AT-Glu administration, decreased speed of movement was observed. Quantitative evaluation of monoamine level in the hippocampus and prefrontal cortex revealed the main neurochemical changes under the influence of AT-Glu, probably associated with memory formation, only in the hippocampus, and they were related to the DAergic system. The role of the DAergic system in the mechanisms of learning and memory regulation has been thoroughly studied [8,10], the decrease in DA content in the hippocampus, the structure directly involved in spatial memory formation, can probably be explained by increased DA expenditure for nervous system activation during learning. At the same time, AT-Glu had no pronounced effect on amino acid pattern in this structure, in contrast to the frontal cortex. The detected stimulatory effect of AT-Glu on all studied neurotransmitter amino acids, including glutamate, in this brain structure can be explained by the cooperative effect of AT-Glu and CPAR procedure associated with animal fear. Thus, AT-Glu administered to aging C57Bl/6 mice had a protective effect and prevented age-related memory impairment.

REFERENCES

1. Gorbatov VY, Trekova NA, Fomina VG, Davydova TV. Anti-amnesic effects of antibodies to glutamate in experimental Alzheimer's disease. *Bull. Exp. Biol. Med.* 2010;150(1):23-25.
2. Gruden MA, Davydova TV, Kudrin VS, Narkevich VB, Vetrile LA, Morozova-Roche LA, Sewell RDE. Neuroprotective effects of glutamate antibodies on memory impairment induced by proinflammatory S100A9 protein oligomers in aging animals 5. *Patol. Fiziol. Eksp. Ter.* 2017;61(4):13-20. Russian.
3. Gruden MA, Davydova TV, Fomina VG, Vetrile LA, Morozova-Roche LA, Sewell RD. Antibodies to Glutamate Reversed the Amnesic Effects of Proinflammatory S100A9 Protein Fibrils in Aged C57Bl/6 Mice. *Bull. Exp. Biol. Med.* 2017;162(4):430-432.
4. Kolobov VV, Davydova TV, Fomina VG. Protective action of glutamate antibodies on increased expression of genes of programmed death of rat brain cells induced by injection of a β -amyloid fragment (25-35). *Biol. Bull.* 2014;41(2):118-125.
5. Kolobov VV, Fomina VG, Davydova TV. Antibodies to glutamate reduce the neurotoxic effects of A β 25-35 in prefrontal cortex cell transcriptome. *Dokl. Biochem. Biophys.* 2012; 447(1):277-279.
6. Davydova TV, Barashova LA, Kovalev IE, Evseev VA. Suppression of alcohol consumption by the induction of the synthesis of serotonin antibodies. *Farmakol. Toksikol.* 1987;50(2):57- 58.
7. Gruden MA, Davydova TV, Wang C, Narkevich VB, Fomina V.G, Kudrin VS, Morozova-Roche LA, Sewell RD. The misfolded pro-inflammatory protein S100A9 disrupts memory via neurochemical remodelling instigating an Alzheimer's disease-like cognitive deficit. *Behav. Brain Res.* 2016;306:106-116.
8. Kushida S, Kimoto K, Hori N, Toyoda M, Karasawa N, Yamamoto T, Kojo A, Onozuka M. Soft-diet feeding decreases dopamine release and impairs aversion learning in Alzheimer model rats. *Neurosci. Lett.* 2008;439(2):208-211.
9. Pearson SJ, Czudek C, Mercer K, Reynolds GP. Electrochemical detection of human brain transmitter amino acids by high-performance liquid chromatography of stable o-phthalaldehyde-sulphite derivatives. *J. Neural Transm. Gen. Sect.* 1991;86(2):151-157.
10. Puig MV, Rose J, Schmidt R, Freund N. Dopamine modulation of learning and memory in the prefrontal cortex: insights from studies in primates, rodents, and birds. *Front. Neural Circuits.* 2014;8:93. doi: 10.3389/fncir.2014.00093.
11. Seguela P, Geffard M, Buijs RM, Le Moal M. Antibodies against gamma-aminobutyric acid: specificity studies and immunocytochemical results. *Proc. Natl Acad. Sci. USA.* 1984; 81(12):3888-3892.

TABLE 1. Effect of Intranasal AT-Glu Administration on the Content of Biogenic Amines (nmol/g tissue) in the Hippocampus and Frontal Cortex of Aging C57Bl/6 Mice (Mean)

Group	Neurotransmitters and their metabolites										
	DA	DOPAC	HVA	3-MT	DOPAC/DA	HVA/DA	NE	5-HT	5-HIAA	5-HIAA/5-HT	
Hippocampus											
Control	0.821±0.310	0.315±0.053	0.269±0.068	0.045±0.033	0.543±0.300	0.490±0.220	3.477±0.406	5.074±0.524	5.673±1.6	1.103±0.257	
Experiment	0.240±0.038*	0.430±0.054	0.165±0.069*	0.068±0.047*	0.719±0.410*	0.423±0.260	3.536±0.604	5.260±0.790	5.804±1.041	1.026±0.059	
Frontal cortex											
Control	11.994±4.390	0.823±0.259	1.669±0.539	0.553±0.205	0.071±0.007	0.147±0.037	3.406±0.371	4.976±0.485	2.654±0.291	0.589±0.053	
Experiment	13.514±3.592	0.816±0.109	1.0761±0.222	0.739±0.223	0.094±0.004	0.134±0.033	3.604±0.183	5.250±0.333	3.243±0.296	0.621±0.080	

Note. Here and in Table 2. *p<0.05 in comparison with the control.

TABLE 2. Effect of Intranasal AT-Glu Administration on the Level of Neurotransmitter Amino Acids (mmol/g tissue) in the Hippocampus and Frontal Cortex of Aging C57Bl/6 (Mean)

Group	Aspartate	Glutamate	Glycine	Taurine	GABA	Glutamate/GABA
Hippocampus						
Control	1.199±0.200	4.675±0.710	0.652±0.092	7.056±0.965	1.006 ±0.137	4.662±0.461
Experiment	1.160±0.414	4.538±1.632	0.801±0.240	6.732±0.214	1.086±0.373	4.266±0.627
Frontal Cortex						
Control	0.780±0.284	2.614±0.662	0.302±0.055	3.782±1.164	0.517 ±0.136	5.660±0.447
Experiment	1.244±0.429*	4.488±1.649*	0.515±0.181*	6.311±1.258*	0.922±0.397*	4.949±0.207