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Antibodies to glutamate facilitate spatial memory formation in the Morris maze in aging C57BL/6 mice

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Intranasal administration of antibodies to glutamate at dose of 250 μg/kg for two weeks facilitated spatial learning and memory formation in the Morris water maze in aging C57Bl/6 mice. Concurrently, in the animals treated with glutamate antibodies, there was a decrease in serotonin level, no change in dopamine but a reduction in 3-MT and HVA metabolite concentrations in the hippocampus. In the prefrontal cortex, a decrease in dopamine level occurred along with a simultaneous increase in the content of its metabolite, DOPAC, as well as an increase in excitatory and inhibitory amino acid neurotransmitters: aspartic acid, glutamate, glycine, taurine and GABA. It was concluded that Abs-Glu facilitated spatial learning and memory formation through a remodeling of the hippocampal and prefrontal cortical neurochemical system in aging C57Bl/6 mice.

Keywords: aging, memory, antibodies to glutamate, neurotransmitters

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INTRODUCTION

Aging is a physiological process which is accompanied by physical and psychological changes, disruption of homeostasis and increased age-related destabilization of vital functions. It is invariably linked with cognitive deficits, in particular, memory impairment [10]. In this regard, the search for new approaches to prevent and correct age-related changes in short- and long-term memory, as well as understanding the underlying mnestic processes, is a focus of research. An immunological approach to ameliorate cognitive impairment during aging, using antibodies to endogenous substances involved in molecular mechanisms of memory formation, is undoubtedly relevant. In previous studies, we demonstrated that chronic intranasal administration of glutamate antibodies (Abs-Glu) to 12-month old C57Bl/6 mice (250 μg/kg) led to an

improvement in passive avoidance test performance. Moreover, intranasal Abs-Glu did not affect motor activity in the open field test, indicating a selective effect of Abs-Glu on memory formation [2]. Consequently, the aim of this study was to investigate the effects of Abs-Glu on learning and spatial memory in the Morris water maze in relation to hippocampal and prefrontal cortical concentrations of biogenic amines, their metabolites and amino acid neurotransmitters in aging C57Bl/6 mice.

METHODS

The study was conducted on 12-month old male C57Bl/6 mice (n = 24) and weighing 32.2 ± 1.8g (supplied by the Research Institute of General Pathology and Pathophysiology). All experiments with animals were carried out in compliance with the requirements set out in the Directive of the European Parliament and the Council of the European Union 2010/63 / EU of September 22, 2010, as well as in accordance with the rules approved by the Commission on Bioethics, Research Institute of General Pathology and Pathophysiology. The mice were kept under standard vivarium conditions with free access to water and food and maintained on a 12:12 hour light-dark regime.

Abs-Glu were obtained according to the previously described method involving immunization of male chinchilla rabbits (n = 5) with a BSA/glutamate conjugate [2]. The purified polyclonal monospecific Abs-Glu was used in experiments in a titer of 1:1024 ± 1:16.

The animals were divided into 2 groups: the experimental group (n = 12), were intranasally administered (alternately in each nostril) a solution of purified Abs-Glu at a dose of 250 μg/kg, dissolved in 4.0 μl of physiological saline, daily for 14 days. The control group (n = 12), received 4.0 μl of saline daily over the same 14-day period. Twenty-four hours after the last nasal inoculation, behavioral experiments were performed involving training, formation, and testing of spatial memory using the Morris water maze model (Columbus Instruments, USA) according a previously described protocol [8]. The Morris maze consisted of a circular pool (diameter 140 cm, side height 60 cm), painted in light gray and filled with water at a temperature of 22 ± 1.0°C to a height of 40 cm.

Automated video surveillance was performed using the EthoVision System (Noldus Information Technology). During training, a submerged hidden platform with a diameter of 11 cm was placed 1.0 cm below the water level and always in the same place in the water maze. Visual landmarks

(design, images) were located outside the installation of the water maze. Over 4 consecutive training days, each animal was allowed 4 trial attempts to search for the hidden platform, each time placing the mouse in different quadrant of the water maze. The duration allowed for each attempt was 60 s and if the platform was located, the animal was permitted to stay on it for 30 s. The interval between training attempts was 5 minutes. On experimental test days, spatial skill training was evaluated using the animal latency period (LP) to reach the platform. Thus, on protocol day 5, spatial memory was tested by gently placing the animal in a quadrant remote from the target hidden platform and then recording the LP for an allotted 60 s period.

Twenty-four hours after behavioral experiments, all animals were decapitated then hippocampal and prefrontal cortical brain structures were dissected on ice (4 °C). The following biogenic amine content was determined in the extracted samples of cerebral structures: dopamine (DA), serotonin (5-HT), norepinephrine (NA), in addition to the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 3-methoxytyramine (3-MT) along with the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA) as well as amino acid neurotransmitters: aspartate (Asp), glutamate (Glu), glycine (Gly), taurine (Tau) and γ-aminobutyric acid (GABA).

Brain region neurotransmitter concentrations were determined by HPLC with electrochemical detection (HPLC/ED) using LC-304T chromatograph (BASi) with a Rheodyne 7125 injector and the sample loop volume was 20 μl [1,4]. The concentration of monoamines (nmol/g of tissue) in the experimental samples was calculated by the internal standard method, based on the ratio of the peak areas in the standard mixture and in the experimental sample. The content of neurotransmitter amino acids was determined by HPLC/ED according to the standard method [3,5]. A mixture of aspartate, glutamate, taurine and GABA at a concentration of 0.1 μM in 0.1 N. $HClO_4$ was used as a standard solution for calibrating the sorbent column. The concentration of amino acid neurotransmitters was expressed in mmol/g of tissue.
The data obtained were statistically analysed using the Statistica 6.0 program (StatSoft, Inc.) using intergroup comparison by the Mann-Whitney U test.

RESULTS AND DISCUSSION

In the first 3 days of spatial navigation training, the mean latency for platform location (LP) did not differ significantly between groups (figure 1). However, on the 3rd training day, significant differences were found in the platform location LP during the 3rd and 4th attempts between the

Abs-Glu experimental and control groups. Thus, the platform LPs (3rd and 4th attempts) for the experimental group were respectively 36.8 ± 6.1 s and 33.6 ± 5.9 s, in contrast to 50.4 ± 6.6 s and 50.2 ± 6.5 s in the control group ($p < 0.05$). On the 4th training day, the mean LP of the Abs-Glu treated experimental mice was significantly decreased in comparison to the controls (figure 1). On the test day, the mean platform LP was significantly lower in the Abs-Glu treated group (32.5 ± 7.7 s) compared with the controls (54.6 ± 5.4 s; $p = 0.035$) indicating an improvement in long term memory.

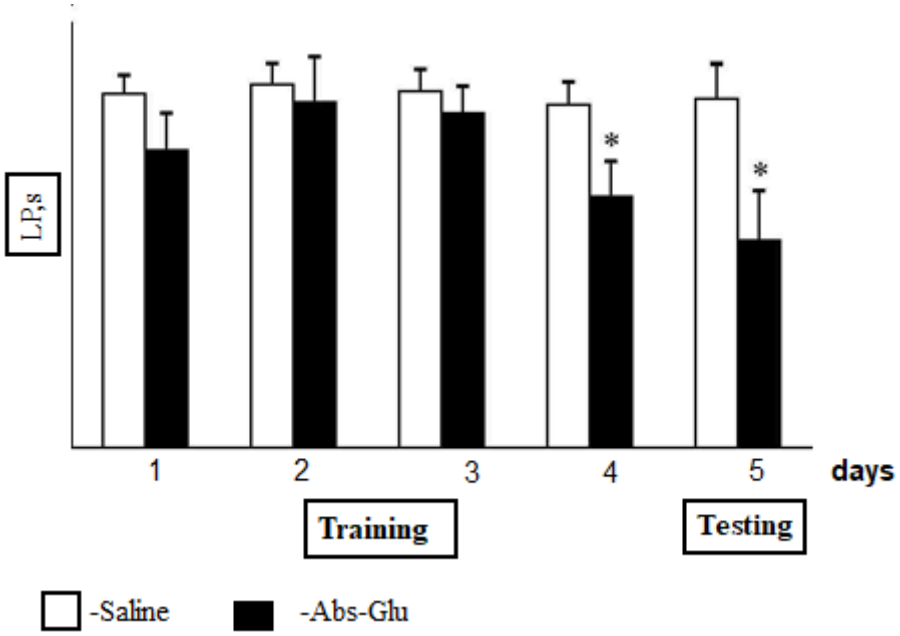

Figure 1. Group latency period (LP) platform location in the Morris water maze by aging C57Bl/6 mice (mean value of 4 attempts) following chronic intranasal administration of Abs-Glu in comparison with control saline treatment. * $p < 0.05$ compared with the control (U Mann – Whitney test).

Analysis of other parameters in the Morris water maze did not reveal any differences in the cumulative swimming distance traveled or the speed. Thus, the mean total swim distance during training in the Abs-Glu experimental group was 676.1 ± 144.4 cm and 632.3 ± 104.8 cm in the controls, while the swim speed in these groups was 13.3 ± 1.8 and 13.4 ± 1.4 cm/s respectively. Overall, the findings indicate that chronic intranasal administration of Abs-Glu to aging mice significantly facilitates spatial learning and memory in the Morris water maze.

It is known that for the process of memorizing new information (i.e. in this instance, location of the hidden platform), the hippocampus and prefrontal cortex areas of the brain are involved. The hippocampus is responsible for the processing and storage of recently acquired information, and

the prefrontal cortex provides a long-term storage function for memory. However, the question arises how these brain areas interact in the realization of the integrative brain activity related to learning and memory [9,10]. In this context, the dorsolateral component of the prefrontal cortex is considered to be a focus of visual spatial memory [6]. Hence, we performed a neurochemical analysis, which revealed a concentration profile for monoamines and their metabolites in the hippocampus and the prefrontal cortex of mice treated intranasally with Abs-Glu (Table 1).

Table 1. The hippocampal and prefrontal cortical content of neurotransmitters and their metabolites (nmol/g tissue) in aging C57Bl/6 mice with chronic intranasal administration of Abs-Glu and saline

Animal group	NA	DA	DOPAC	HVA	3-MT	5-HT	5-HIAA
Hippocampus							
Saline	3.66±0.47	0.29±0.19	0.76±0.36	0.61±0.26	0.49±0.37	0.41±0.20	0.38±0.23
Abs-Glu	3.82±0.89	0.33±0.18	0.74±0.25	0.46±0.16*	0.16±0.070*	0.13±0.06*	0.35±0.09
Prefrontal cortex							
Saline	2.94±0.54	3.20±0.60	0.56±0.24	1.88±0.70	0.55±0.11	0.60±0.33	0.59±0.21
Abs-Glu	2.49±1.11	0.29±0.13*	1.33±0.39*	0.76±0.07	0.53±0.25	0.59±0.26	0.69±0.11

* $p < 0.05$ compared with the control (U Mann – Whitney test).

It was shown that in the hippocampus of animals administered Abs-Glu, the content of NA, DA and DOPAC remained unchanged, but the concentration of 3-MT and HVA decreased significantly ($p = 0.05$) by 67.3% and 26.6%, respectively. This finding may well have reflected an inhibition of enzymes involved in the metabolism of catecholamines, i.e. catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO). Concomitantly, the DOPAC/DA ratio was comparable the control value but the HVA/DA ratio diminished by 35.6% (Table 2).

Table 2. Metabolism of neurotransmitters and their metabolites (conventional units) in the hippocampus and prefrontal cortex in aging C57Bl/6 mice with chronic intranasal administration of Abs-Glu.

Animal group	DOPAC/DA	HVA/DA	5-HIAA/5-HT
Hippocampus			
Saline	3.62±0.75	2.68±0.83	1.98±0.82
Abs-Glu	3.52±0.51	1.73±0.81*	3.71±1.29*
Prefrontal cortex			
Saline	0.30±0.33	0.53±0.29	4.25±1.61
Abs-Glu	3.82±0.76*	3.11±0.82*	1.42±0.62*

It was also found that in the hippocampus, the 5-HT concentration following Abs-Glu treatment was significantly reduced ($p = 0.05$) by 68.3% in the absence of any apparent change in 5-HIAA level but the 5-HIAA/5-HT ratio was augmented 187.3% ($p = 0.01$) (Table. 2). It may be conceivable from this outcome, that a pronounced decrease in hippocampal 5-HT concentration is associated with an activation of its binding to 5-HT receptors though there was some evidence of an increase in 5-HT turnover.

Chronic administration of Abs-Glu decreased the concentration of DA in the prefrontal cortex by 90.9% ($p = 0.05$), while the content of its metabolite, DOPAC, significantly increased by 237.5% ($p = 0.01$). In contrast, the HVA metabolite level decreased by 56.9% ($p = 0.05$) but the concentration of 3-MT did not change, which, taken as a whole, is indicative of a selective enzyme activation of DA metabolism. The data on the amino acid neurotransmitter levels in response to ABS-Glu are presented Table 3.

Table 3. The content of neurotransmitter amino acids (mmol/g of tissue) in the hippocampus and prefrontal cortex in aging C57BL/6 mice with chronic intranasal administration of Abs-Glu.

Animal group	Asp	Glu	Gly	Tau	GABA
Hippocampus					
Saline	1.83±0.31	5.68±0.95	0.50±0.05	5.22±0.74	2.72±0.60
Abs-Glu	1.57 ±0.54	4.74±1.71	0.42±0.07	4.79±1.51	2.77±0.85
Prefrontal cortex					
	0.61 ±0.163	1.31±0.35	0.33±0.12	1.75±0.40	0.48±0.23
	1.82±0.56*	3.65±0.25*	0.54±0.06*	4.73±1.00*	2.25±0.63*

* $p < 0.05$ compared with the control (U Mann – Whitney test).

The main effects of chronic administration of Abs-Glu are focused not in the hippocampus, but in the prefrontal cortex, although a slight (16.6%) decrease in the concentration of glutamate was observed in the hippocampus. Thus, in the prefrontal cortex, a contrasting increase in the concentration of both excitatory and inhibitory amino acids was found: aspartic acid by 98.3%, glutamate by 78.6% ($p = 0.05$), glycine by 63.5%, taurine by 70.9% ($p = 0.05$) and GABA by 368.8% ($p = 0.01$). It could be assumed that such effects of Abs-Glu on the metabolism of amino acid neurotransmitters in the prefrontal cortex is associated with a general activation of their synthesis in the aging brain during spatial skill learning. On the one hand, this conclusion would account for the unilateral excitatory/inhibitory promotion of amino acids and on the other, it rationalizes an element underlying the process of remembering the hidden platform location. In concurrence with this deduction, taurine is not only known to improve spatial learning and memory [7] but it has also been reported to beneficially increase hippocampal neurogenesis in the context of brain aging [11]. An increase in the content of glutamate under the influence of Abs-Glu signifies improved neurotransmission while facilitating the formation of spatial memory in these aging animals. In addition to taurine and glutamate, the increased content of the inhibitory neurotransmitter GABA, indicates the formation of a specific neurochemical configuration in the prefrontal cortex, leading to enriched learning and the consolidation of spatial memory during aging. Thus, we can conclude that Abs-Glu promote spatial learning and memory formation through a remodeling of the hippocampal and prefrontal cortical neurochemical system in aging C57Bl/6 mice.

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