Effects of combined substance membraton on correction of the indicators of zoo-social behavior of old rats

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Abstract. Brain aging is accompanied by the development of an imbalance of the systems of chemical regulation of cells, a decrease in neurotrophic factors, and disorders of brain plasticity. Important place in the neurotransmitter changes in aging is given to the γ -aminobutyric acid (GABA), represented in the system of both external and internal neurotransmitters. With aging, the activity of GABA systems changes and the content of GABA in brain structures decreases, which is associated with impaired mental function and the development of pathology, in particular, dementia. Given the prevalence of pathology and the rate of population aging, the search for effective and safe means for the prevention and comprehensive metabolic treatment of age-related cognitive and psycho-emotional disorders remains relevant. In experiments on adult (10 months) and old (24 months) *Wistar* male rats, we investigated the pharmacological activity and age-specificities of the combination, including GABA and magnesium gluconate in the form of a coordination compound (γ -aminobutyro-Mg(II)-gluconate), and pyridoxine hydrochloride (Membraton). Tests "dark / light camera" and "three-chamber activity" established age differences in the behavior of rats. In older animals, amid increased anxiety, there was a decrease in zoo social interaction, such as communication, novelty preference, and social recognition. Under the influence of the course introduction of Membraton (100 and 500 mg / kg) in old rats, anxiety decreased and social activity increased: motivation and initiation of social contact increased, contact with an unfamiliar partner-stimulus increased, recognition of a familiar and unfamiliar partner was normalized. Under the influence of the remedy, indicators of social behavior of old animals approached the level of young animals. The established effects make it possible to consider Membraton as a promising metabolic therapy for increasing sociality in psycho-emotional disorders associated with age.

Key words: Membraton, zoo social activity, communicativeness, social recognition, anxiety, aging.

Reduction of cerebral circulation, decrease in the number of nerve cells, decrease in the content of transmitters and sensitivity of receptors to them, structural and functional changes of synaptic membranes are the main factors that accompany the aging of the brain and cause disturbances of its integrative function [1-4]. Involutional processes in the body, along with physical and mental functions, determine the condition of the so-called "mental aging" [5]. Such a condition is characterized by a decrease in the level of mental activity, slowing of psychomotor reactions, narrowing of volume and difficulty of perception of new information, memory impairment, emotional disorders, inadequacy of reactions to external and internal stimuli [6]. One of the consequences of aging is a decrease in social activity, which disrupts relationships and impairs the quality of life of older people. However, the support of any form of social activity during aging has a positive effect on the psyche, helps to increase the adaptive capacity of the body and prolong the active period of life [5, 7]. Therefore, the search for new safe methods and means of correcting mechanisms related to the development of social deficiency in

aging remains relevant. However, the development of such tools is complicated by the lack of adequate experimental models of behavioral pathology that would allow successful pharmacological screening of psychotropic agents in conditions as close to clinical as possible.

It should be noted that complex behaviors, both social (social memory and social recognition, sexual and family behavior, social advantage, interspecific interaction), and non-social (learning, remembering) in animals also undergo a wide range of changes in aging [8]. Decreased sensory and motor functions and impaired coordination [9], increased anxiety [10], cognitive dysfunction associated with a gradual decrease in structural and functional plasticity in the hippocampus and prefrontal cortex [11] - these are just the main factors that influence the behavior of animals. Changes in metabolic and physiological processes that occur with aging lead to a progressive decrease in resistance to stress, impaired perception and processing of information that enters and determines the type of behavior, worsen zoo-psychic functions and alter the social activity of animals [8, 12]. These changes in mechanisms, symptoms and pathophysiological manifestations are recognized as similar to pathology in humans [13-15]. Therefore, spontaneously aging animals can rightly be used as models to study the corrective effects of new pharmacological agents on psycho-emotional activity and behavior.

In view of the above, the aim of the study was to experimentally study the features of the zoo social interaction of old animals and the effect on it of a new combination agent Membraton, including γ -aminobutyric acid (GABA), in the form of a magnesium gluconate coordination compound and pyridoxine hydrochloride.

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Material and methods

The studies were performed on *Wistar* rats of adult (10 months) and old (24 months) age, obtained from the experimental-biological clinic of the D.F. Chebotarev State Institute of Gerontology of the NAMS of Ukraine. Each age group consisted of 30 animals, divided into three subgroups: 1 – control group, 2 and 3 - animals receiving Membraton intragastrically (with feed) at the rate of 100 and 500 mg/kg. The course of administration of the drug was 21 days.

To investigate age-specific features of social behavior and identify the effects of Membraton on it, a three-chamber activity test was used to assess sociability, recognition, and preference for social novelty. The test is widely used, in particular, both in the modeling of psycho-emotional disorders and in the study of neural mechanisms that influence social behavior [16, 17]. The studies were carried out in a special plastic chamber with transparent walls separated by partitions into three identical compartments. In the partitions, openings-entrances were made for the free movement of rats between the compartments of the chamber, and the free choice of the animal of one of the three compartments to contact or avoid a partner. The experiment consisted of three 10-minute sessions. In the first session, the animal was placed for adaptation in the central compartment of the chamber, from which it could move freely into the left or right compartments. In the second session in the left compartment was placed an unfamiliar rat (stimulus S1), "immobilized" in a container of metal mesh, and the test animal was placed in the central compartment, we recorded the time of exit from the center and the time of staying in the left, center or right compartment. In this session, communicability was assessed as a tendency to be near the stimulus animal rather than in identical empty compartments. In the third session, a new "immobilized" unfamiliar rat (stimulus S2) was placed in the right compartment, so that stimulus S1 became already familiar, and the same indicators as in the second session were recorded, assessing the ability to recognize and prefer social novelty. The method is based on the fact that normally in socially active animals with the appearance of a new rat stimulus, the process of "social novelty" prevails, a rat prefers the study of a new social object and loses interest in an already familiar partner. Prior to the test, stimulus rats did not contact experimental animals. After each session, the camera was thoroughly wiped with a damp and dry cloth.

Rat anxiety was assessed using a specific «dark / light camera» test [18]. The animal was placed in a light chamber and for 4 min the latent time of approach to the dark chamber, the number of movements

between the chambers, the time in the light and dark chambers, and the number of vertical risings were recorded as an indicator of research activity.

Statistical analysis of the obtained results was performed using Student's t-test (the data presented as $M \pm m$) and Wilcoxon-Mann-Whitney test for nonparametric samples (the data are presented as median, minimum and maximum values) [19].

Results and their discussion

Analysis of the data obtained in the social activity tests revealed clear age differences in communication indicators (Fig. 1).

Figure 1. Distribution of time spent in the center, with an unfamiliar partner (S1) and in an empty chamber during the 2 sessions of the communicative test

Notes: * - $P < 0.05$ compared to young rats; # - $P < 0.05$ compared to control rats of the appropriate age

Thus, during the second session, the adult rats of the control group quickly left the center and ($40 \pm 9\%$) of the time spent exploring the unfamiliar rat S1 stimulus. Old animals spent only ($19 \pm 7\%$) of the time on an unfamiliar S1 stimulus ($P < 0.05$), while the rest of the time were in the center and in the empty chamber. The number of sensory contacts with S1 stimulus in control adults and old rats also differed significantly, being 7 (3... 12) and 0 (0... 1), respectively, $P < 0.01$. It should be noted that the stay in the central chamber indicates increased anxiety and decreased research activity, and lack of contact with an unknown partner - a low level of initiation and avoidance of social contact in old animals.

In experimental rats receiving Membraton (100 mg / kg), the stay time with S1 stimulus statistically significantly increased: in adults - 1.9 times, in the elderly - 2.8 times, which almost corresponded to the index of adult animals and was due to a statistically significant reduction in the empty space. The number of sensory contacts in the experimental adult rats remained practically unchanged at 9 (7... 11), in the old ones it increased to 5 (0... 10), which was also not statistically different from the indicator of adult animals. Increasing the Membraton dose to 500 mg/kg did not increase the effect, but, as in the lower dose, the remedy eliminated age-related differences in the communicative rate in older animals.

During the third test session, the ability of rats to identify a new partner and the preference for novelty were assessed (Fig. 2).

Figure 2. Distribution of time spent in the center, with a familiar partner (S1) and a new partner (S2) during the 3 sessions of the social novelty test

Notes: * - $P < 0.05$ compared to young rats; # - $P < 0.05$ compared to control rats of the appropriate age

It was found that the adult control animals had a pronounced preference for the new S2 partner over the already familiar S1: with the new S2 stimulus, adult animals spent $(85 \pm 7)\%$ of the time, whereas with the S1 stimulus only $(11 \pm 1)\%$. Unlike adults, old control rats more than half the time $(56 \pm 17)\%$ remained in the center of the installation, and the remaining time was equally $(22 \pm 16)\%$ and $(22 \pm 13)\%$ distributed between familiar S1 and unfamiliar S2 partners. There were no sensory contacts with the familiar S1 stimulus in adult rats, and they contacted 8 (3... 10) times with the new S2 stimulus, whereas in old rats the number of contacts with S1 and S2 stimuli was statistically smaller being in both cases 0 (0 ... 2), which indicates a loss of recognition ability.

The administration of Membraton practically did not affect the normal recognition rates in adult animals. In old rats, when injected at a dose of 100 mg/kg, there was a slight increase in stay time with stimuli both S1 and S2 due to a reduction in the proportion of time spent in the central compartment of the chamber. Following the administration of 500 mg/kg of Membraton in old rats, the stay time with an unfamiliar S2 stimulus increased 2.6 times and almost reached the level of young animals. The number of sensory contacts with S2 in them also increased from 0 (0...2) to 4 (2... 5), which was not statistically different from that in adult rats, indicating a clear preference for the new stimulus and improved ability to recognize under the influence of the drug.

Therefore, the results of the test indicate a decrease in social activity in old animals and are consistent with the literature [8]. The combined drug Membraton reduced age-related differences in social interaction indicators as one of the components of the brain's integrative function, and improved communication and social recognition in older animals. The remedial effects of Membraton on zoo social behavior are likely to be associated with a reduction in anxiety, which plays a significant role in emotional and cognitive impairment. To confirm the anxiolytic component in the spectrum of pharmacological activity of Membraton, studies were conducted using the specific method "dark / light camera". It was found that in the latent time of the first approach to the dark chamber and the time in the light and dark chambers, control adults and old rats did not differ significantly: animals of both age

groups spent less time in the light chamber and, accordingly, spent more time in the dark chamber, however, the number of steps to the dark camera in the old rats was statistically higher than in adults (Tab. 1).

Table 1

Indicators of anxiety in adults and old rats under the influence of membratone ($M \pm m$)

Group rats	The age of rats, months	Latent sunset time, sec	Stay time in the cameras, sec		Number of camera visits		Number of vertical risings
			light	dark	light	dark	
Control	10	13.2 $\pm$ 3.9	26.7 $\pm$ 5.2	273.3 $\pm$ 5.2	2.4 $\pm$ 0.4	2.3 $\pm$ 0.3	2.0 $\pm$ 0.7
	24	20.4 $\pm$ 4.4	37.0 $\pm$ 6.9	263.8 $\pm$ 6.6	2.5 $\pm$ 0.5	3.6 $\pm$ 0.5 *	2.3 $\pm$ 0.7
Membraton, 100 mg/kg	10	22.0 $\pm$ 5.5	25.0 $\pm$ 0.2	249.1 $\pm$ 8.1 #	3.9 $\pm$ 0.4 #	3.8 $\pm$ 0.4 #	4.6 $\pm$ 0.8 #
	24	26.0 $\pm$ 5.2	84.0 $\pm$ 15.9 *, #	216.0 $\pm$ 15.9 #	4.1 $\pm$ 0.4 #	5.1 $\pm$ 0.4 #	5.0 $\pm$ 1.8
Membraton, 500 mg/kg	10	31.7 $\pm$ 4.3 #	49.9 $\pm$ 4.5 #	250.1 $\pm$ 4.5 #	3.3 $\pm$ 0.4	3.3 $\pm$ 0.4	3.1 $\pm$ 0.6
	24	35.8 $\pm$ 5.9 #	84.5 $\pm$ 16.3 #	216.8 $\pm$ 16.5 #	3.3 $\pm$ 0.6	4.3 $\pm$ 0.6	4.3 $\pm$ 0.5 #

Notes: * - $P < 0.05$ compared to young animals; # - $P < 0.05$ compared to age-matched controls.

After the administration of Membraton (100 mg / kg) in old rats, the stay time in the light chamber was significantly increased, the time spent in the dark chamber decreased, the number of entries both to the light and the dark chamber increased, as well as the number of vertical risings compared to the control animals of the same age. In addition, at a dose of 500 mg/kg, Membraton significantly increased the latent time of entry in the dark chamber, which generally indicates a decrease in anxiety and a positive effect on locomotor and research activity of old rats. Similar changes in behavior in the "dark / light camera" were observed in adult rats. Therefore, Membraton has an anxiolytic effect, one of the consequences of which is the normalization of the zoo social activity of old animals.

The psychoneurotropic effect of Membraton is obviously caused by the complex action of the active components that make up its composition - GABA, magnesium gluconate and vitamin B6. Thus, GABA, as a universal inhibitory neurotransmitter, regulates numerous processes in the body, from muscle tone to emotional reactions, including the formation of integrative functions of the brain, has nootropic action [20, 21]. Magnesium decreases excitability and weakens emotional manifestations, independently stimulates the activity of central GABA-ergic systems, as a modulator of GABA receptors increases their activity and causes anxiolytic effect [22, 23]. Magnesium deficiency has been linked to many age-dependent diseases [24]. At the molecular-receptor level, it has been shown that an increase in magnesium in brain tissue leads to an increase in synaptic plasticity and an improvement in learning and memory function in old rats [25]. Magnesium is functionally linked to Vitamin B6, which helps to increase the intracellular content of this trace element. In addition, vitamin B6 is known for its neuroprotective and antioxidant activity, is involved in the synthesis of neurotransmitters, including GABA, affects the inhibitory processes in the CNS, stimulates the natural mechanisms of restoration of nervous tissue function [26]. Pyridoxine has traditionally been used to treat cognitive impairment, as it is capable of affecting concomitant symptoms such as asthenia, increased sensitivity to stress, changes in emotional background, anxiety [2, 28]. The chelate (coordination) form of the components of Membraton

allows to preserve biological activity and to improve pharmacokinetic characteristics, which is an advantage of the drug.

Thus, assessing the overall pharmacological effect of the new combination agent, it can be assumed that its corrective effects on social activity and anxiolytic action are mainly related to the restoration of the balance between excitatory and inhibitory processes, which allows to optimize brain function in aging. The established effects testify to the prospect of practical use of Membraton, as a means of complex metabolic therapy, to increase sociality in psycho-emotional disorders in the elderly.

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References

1. Stareniye mozga [Brain aging]. Frolkis, V.V., Ed. The science (in Russian): Leningrad, **1991**; p 277.
2. Mattson, M.P. & Arumugam, T.V. Hallmarks of brain aging: adaptive and pathological modification by metabolic states. *Cell. Metab.* **2018**, 27, pp. 1176–99. doi: 10.1016/j.cmet.2018.05.011
3. Mostafavi, S.; Gaiteri, C.; Sullivan, S.E. et al. A molecular network of the aging human brain provides insights into the pathology and cognitive decline of Alzheimer's disease. *Nat. Neurosci.* **2018**, 21, pp. 811–19. doi: 10.1038/s41593-018-0154-9
4. Elliott, M.L.; Belsky, D.W.; Knodt, A.R. et al. Brain-age in midlife is associated with accelerated biological aging and cognitive decline in a longitudinal birth cohort. *Mol Psychiatry* **2019**, 10, p 1-10 doi:10.1038/s41380-019-0626-7
5. Stewart-Hamilton, I. *The Psychology of Ageing. 4th edition*. Peter (in Russian): St. Petersburg, **2010**; p 320.
6. Raevsky, A.A. Struktura psikhicheskogo sostoyaniya pozhilykh patsiyentov s organicheskimi zabolevaniyami golovnog mozga i depressivnymi narusheniyami [The structure of the mental state of elderly patients with organic brain diseases and depressive disorders]. Dissertation for scientific degree Candidate of Psychological Sciences: St. Petersburg, **2013**; p 283.
7. Mischikha, L.P. *Psykhologichni osoblyvosti rozvytku osobystosti v period heterohenezu* [Psychological features of personality development in the period of heterogeneity]. Collection of scientific works: philosophy, sociology, psychology (in Ukraine) Play: Ivano-Frankivsk, **2009**, 14, 2; pp. 17–26.
8. Perkins, A.E.; Doremus-Fitzwater, T.L.; Spencer, R.L. et al. A working model for the assessment of disruptions in social behavior among aged rats: The role of sex differences, social recognition, and sensorimotor processes. *Exp. Gerontol.* **2016**, 76, pp. 46–57. doi:10.1016/j.e[ger.2016.01.012.
9. Altun, M.; Bergman, E.; Edström, E. et al. Behavioral impairments of the aging rat. *Physiol. Behav.* **2007**, 92(5); pp 911–23.
10. Belviranli, M.; Atalik, K.E.; Okudan, N. & Gökbek, H. Age and sex affect spatial and emotional behaviors in rats: the role of repeated elevated plus maze test. *Neuroscience* **2012**, 227; pp. 1-9. doi: 10.1016/j.neuroscience.2012.09.036.
11. Bisaz, R.; Boadas-Vaello, P.; Genoux, D. & Sandi, C. Age-related cognitive impairments in mice with a conditional ablation of the neural cell adhesion molecule. *Learn. Mem.* **2013**, 20; pp. 183-93.
12. Shoji, H.; Takao, K.; Hattori, S. & Miyakawa, T. Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. *Mol Brain* **2016**, 9; p 11.
13. McFarland, D. *Animal behavior. Psychobiology, ethology and evolution (in Russian)*. Mir: Moscow, **1988**, p 520.
14. Shoji, H.; Mizoguchi, K. Aging-related changes in the effects of social isolation on social behavior in rats. *Physiol. Behav.* **2011**, 102(1); pp. 58–62.
15. Anisimov, V.N. Molekulyarnyye i fiziologicheskiye mekhanizmy stareniya [Molecular and physiological mechanisms of aging]. The science (in Russian): St. Petersburg, **2003**; p 468.
16. Moy, S.S.; Nadler, J.J.; Perez, A. et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes Brain Behav* **2004**, 3; pp. 287–302.
17. Nadler, J.J.; Moy, S.S.; Dold, G. et al. Automated apparatus for quantitation of social approach behaviors in mice. *Genes Brain Behav* **2004**, 3; pp. 303–14.
18. Bourin, M.; Hascoe, M. The mouse light/dark box test. *Eur. J. Pharmacol.* **2003**, 463; pp. 55– 65.
19. Glanz, S. *Biomedical statistics (in Russian)*. Praktika: Moscow, 1999, p 459.
20. Nuss, Ph.; Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatr. Dis .Treat.* **2015**, 11; pp. 165–75.
21. Nutt, D.J. & Malizia, A.L. New insights into the role of the GABAA —benzodiazepine receptor in psychiatric disorder. *Brit. J.Psychiatry* **2001**, 179(5); pp. 390–96.
22. Vink, R. & Nechifor, M. Magnesium in the central nervous system. *Adelaide: University of Adelaide Press* **2011**, p 342.

23. Eby, G.A.; Eby, K.L. Magnesium for treatment-resistant depression: a review and hypothesis. *Med. Hypotheses* **2010**, *74*(4); pp. 649–60.
24. Barbagallo, M.; Bervedere, M.; Domingues, L.J. Magnesium homeostasis and aging. *Magnesium Res.* **2009**, *22*(4); pp. 235–46.
25. Slutsky, I.; Abumaria, N.; Wu, L.J. et al. Enhancement of learning and memory by elevating brain magnesium. *Neuron* **2010**, *65*(2); pp. 165–77.
26. Mooney, S.; Leuendorf, J.E.; Hendrickson, C. & Hellmann, H. Vitamin B6: a long known compound of surprising complexity. *Molecules* **2009**, *14*(1); pp. 329–51.
27. Shiloh, R.; Weizman, A.; Weizer, N. et al. Antidepressive effect of pyridoxine (vitamin B6) in neuroleptic-treated schizophrenic patients with co-morbid minor depression—preliminary open-label trial. *Harefuah*, *140*(5); pp. 369–73, p 456.
28. Pouteau, E.; Kabir-Ahmadi, M.; Noah, L. et al. Superiority of magnesium and vitamin B6 over magnesium alone on severe stress in healthy adults with low magnesemia: A randomized, single-blind clinical trial. *PLoS One* **2018**, *13*(12); p e0208454. doi: 10.1371/journal.pone.0208454. eCollection 2018.