

Original Article

Peculiarities of behavioral response in rats of different sexes with metabolic syndrome under the conditions of carbacetam administration

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Abstract

Metabolic syndrome has become prevalent worldwide. It is a multi-component, progressing condition closely associated with nervous system pathology. Clinical signs of metabolic syndrome are not often clear, which makes diagnostics more difficult and promotes the development of severe, sometimes irreversible consequences. At the same time, GABA is known to play a considerable role in memorization processes and the mechanisms of memory formation. Thus, early diagnostics and timely treatment improve the prognosis of the disease. Therefore, our work aimed to study the peculiarities of behavioral response and memorization processes of female and male rats with metabolic syndrome under the conditions of carbapenem administration as a modulator of GABA receptors. The experiments were conducted on laboratory nonlinear white male and female rats weighing 0.220–0.250 kg. Metabolic syndrome was simulated by keeping the rats on a high-fat diet and giving them free access to fructose solution (100 g/L). Carbacetam was administered intraperitoneally at 5 mg/kg for 14 days. Conclusions. According to the results of “open field” and “CPAR” tests, both males and females with metabolic syndrome decrease their motor, locomotor, learning and emotional activity. Administration of GABA-receptor modulator motor activity promoted increases and inconsiderable changes in the emotional behavior of rats by the number of washings that occur. The results indicate the experimental rats’ behavior modulation due to carbacetam administration. At the same time, effective retention of conditioned passive avoidance reflex to electric painful stimulation in rats receiving carbacetam indicates improved cognitive ability.

Keywords: metabolic syndrome, carbacetam, cognitive ability of rats.

Introduction

The prevalence of overweight and obesity, together with metabolic syndrome and the development of type 2 diabetes mellitus, increases in the whole world [1]. Metabolic syndrome is known to be a cluster of metabolic anomalies, including obesity and hyperglycemia [2]. It has become a global health care problem occurring in 37.1% in the United States, 10.5% – in Europe,

and over 21.5% – in China [3–5]. The syndrome results from the interrelation of the social environment, behavior and physiological factors. The causes of obesity are very complicated, including genetic, physiological, ecological, behavioral, psychological and even political factors. At the same time, scientific publications available are indicative of considerable gender differences in the occurrence of metabolic syndrome, clinical course and side effects [6, 7].



According to scientific literature data, the functional cycle of gamma-aminobutyric acid (GABA) [8] is associated with glucose metabolism. Certain research demonstrated that oral GABA administration decreases glucose concentration in the blood and improves circulation through insulin increase and glucagon decrease in the blood plasma [9]. At the same time, the functional activity of GABA changes with dysglycemia. However, it is one of the most important mediators of the brain and plays a crucial role in controlling neuron excitability [10]. The majority of GABA-ergic neurons are located in the CNS. Much less of them are found on the periphery. Although many studies have been conducted to learn the role of this amino acid in brain function, its cellular function and physiological importance in other metabolic organs still need to be determined. Moreover, GABA plays a considerable role in memorization processes, the mechanisms of memory formation. Therefore, the study of behavioral response in rats of different sexes with simulated metabolic syndrome and carbacetam administration is relevant.

Our objective is to study the peculiarities of behavioral response and memorization processes in female and male rats with metabolic syndrome under the conditions of carbacetam administration as a modulator of GABA receptors.

Material and methods

The experiments were conducted on laboratory nonlinear white male and female rats with body weights of 0.220–0.250 kg, kept under standard vivarium conditions and natural changes of day and night. The experiments were conducted according to the principles of the Helsinki Declaration on the Humane Treatment of Animals and the European Convention for the Protection of Vertebrate Animals Used in Experiments and for Other Scientific Purposes of 18.03.1986; the EU Directives № 609 f 24.11.1986 and the Order of the Ministry of Health of Ukraine № 690 of 23.09.2009.

Metabolic syndrome was simulated by keeping the rats on a high-fat diet (60 days) enriched by adding solid pork fat and free access to fructose solution (100 g/L) [11]. The simulation of the syndrome was confirmed by the detection of glucose concentration in the blood plasma on an empty stomach and a positive glucose tolerance test. The rats with hyperglycemia lower than 7.0 mmol/L were excluded from the experiment. The group of rats with the syndrome (7 rats) was administered carbapenem intraperitoneally at a dose of 5 mg/kg

for 14 days. The comparison groups, including the control group and the one with simulated pathology (7 rats each), received 0.9% NaCl solution in the same regimen.

The cognitive ability of rats was estimated by the results of the tests "open field" and "conditioned passive avoidance reflex" (CPAR) [12]. The "open field" test was conducted using a camera with plastic walls 40 cm high, and the floor was separated into equal squares with openings imitating holes on the line crosses. Each rat was placed in the center of the camera, and the time of an adjustment period of "immobility" (the latent period) was registered. After that, active behavior for 3 minutes was observed. The following parameters were registered (their number): motor activity – crossed squares; orientation-learning activity – vertical stand, examination of holes; emotional reactions – grooming (washing), fecal boluses (defecation), and urination.

Memory was estimated by "conditioned passive avoidance reflex" (CPAR) using a camera containing a light and dark block connected by an opening imitating a hole. The camera floor in the dark block was connected to an electric current. After the latent period – an interval of staying in the light block – the rats entered the dark one, the opening was closed, and the rats were exposed to stimulation of the limbs with stabilized electric current 0.8 mA for 15 seconds (electric painful stimulation). The time of the latent period was registered since the dynamics of its changes inform about the formation and retaining of the conditioned reflex.

The standard package of statistical analysis programs, Microsoft Excel 2007, was used to process the study's results. The t-student criterion statistically processed the results. Differences were considered reliable with $p < 0.05$. The point estimate of the results was presented in the form of mean values and standard error of the mean ($M \pm m$).

Results and discussion

Considering a generally accepted fact that an "open field" test is highly informative in determining functional changes in the brain, we have analyzed the most important parameters according to the changes in the behavior of rats of both sexes. They include motor activity (number of crossed squares), strategy of learning activity (vertical stands, examination of holes) and the level of emotional response (grooming, defecation, and urination).

Examinations under the conditions of the "open field" test demonstrated (Figure 1 AB and Figure 2 AB)

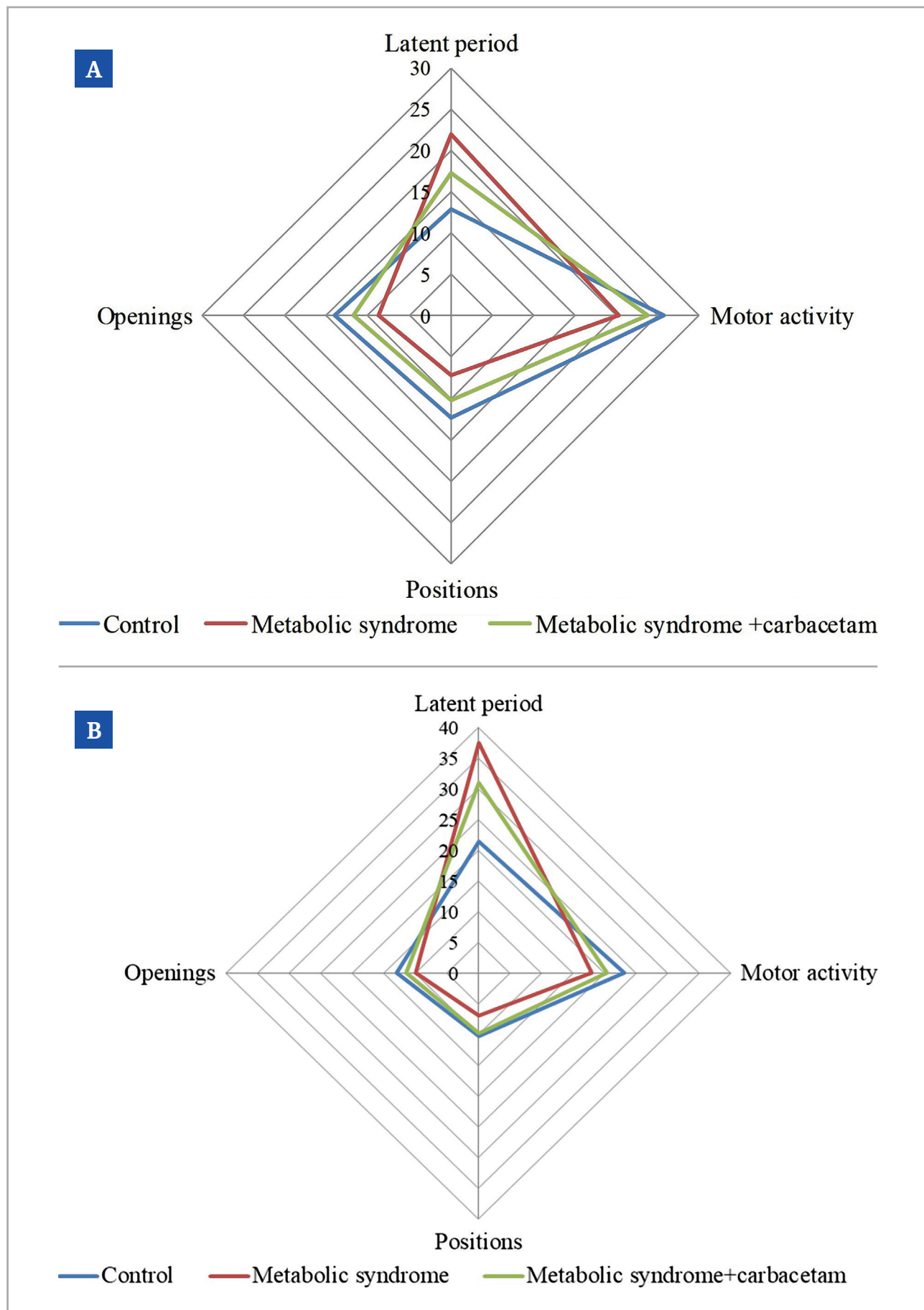


Figure 1: Parameters of motor and learning activity of rats: males (A) and females (B) in "open field" test while stimulating metabolic syndrome under the conditions of carbacetam administration.

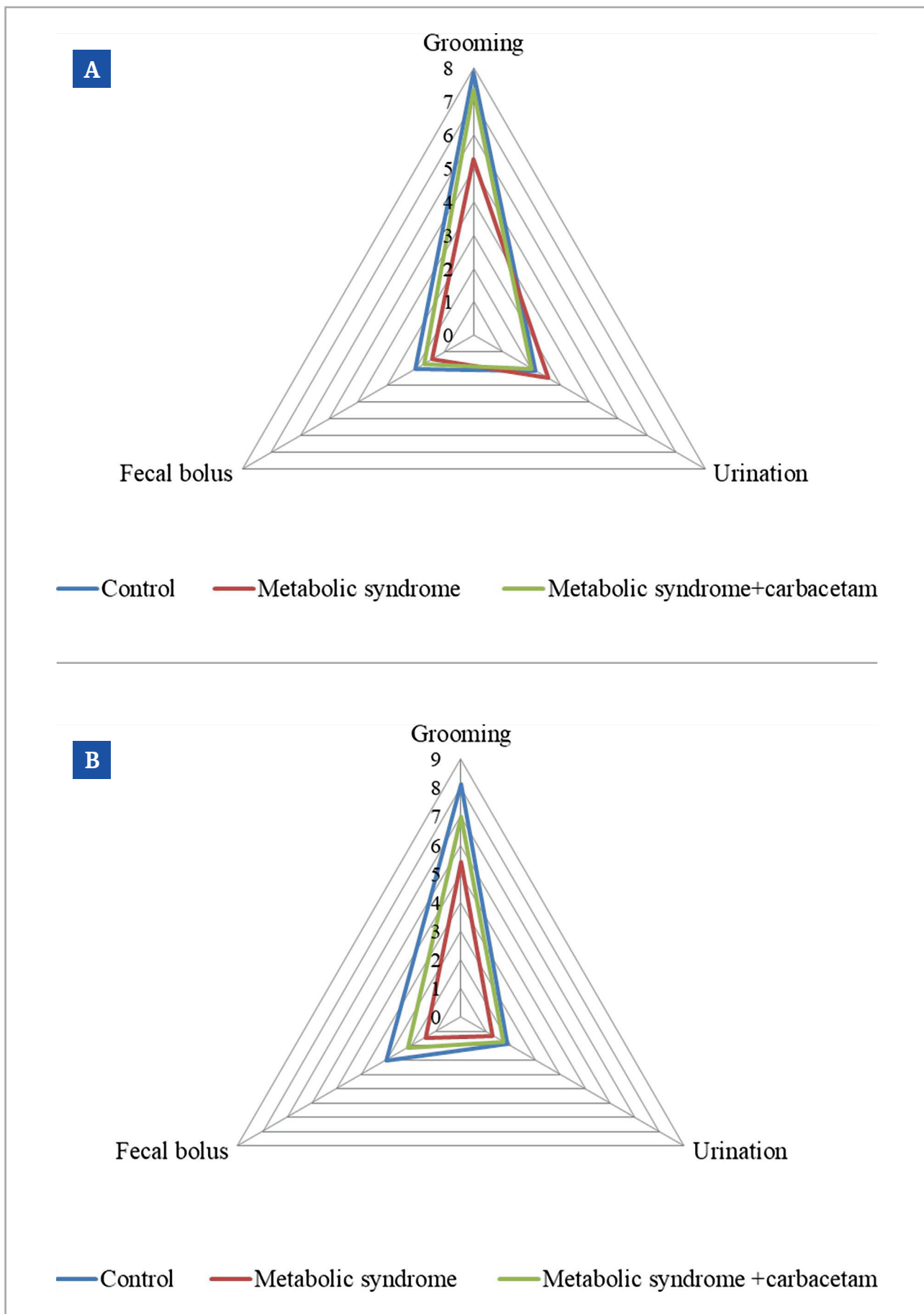


Figure 2: Parameters of the emotional response of rats: males (A) and females (B) in "open field" test while stimulating metabolic syndrome under the conditions of carbacetam administration.

that in rats of both sexes with stimulated syndrome, the latent period increased by 71.8% and 76.6%, respectively. It is longer in females than in males.

Motor activity parameters decreased in the males and females compared with the control group of rats by 21.1% and 22.9%, respectively.

At the same time, the parameters of learning activity decreased as well. Thus, in males, the number of vertical stands and hole learning was 42.3% and 37.9% less, and in females—32.1% and 23.1%, respectively. Analysis of the results demonstrated a decrease of certain parameters in emotional response. Thus, both in

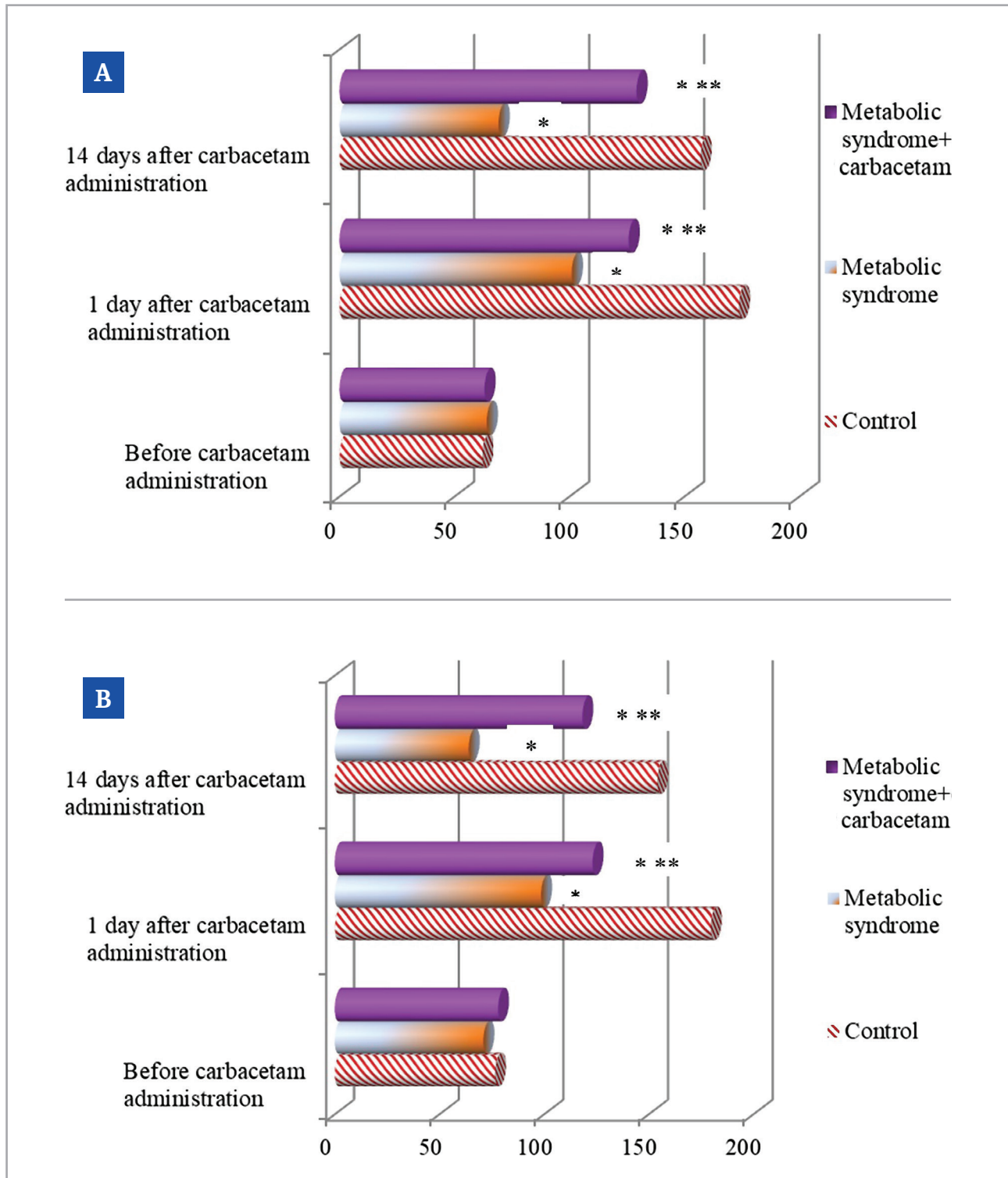


Figure 3: Conditioned passive avoidance reflex test in rats: males (A) and females (B) in "open field" test while stimulating metabolic syndrome under the conditions of carbacetam administration, $M \pm m$. * – statistical significance compared to mean values with the control; ** – statistical significance compared to the metabolic syndrome group.

males and females, the grooming index decreased by 33.1% on average. At the same time, only in females did the amount of fecal boluses decrease by 53.3%.

Thus, rats with stimulated metabolic syndrome demonstrated certain differences in behavior depending on sex. In particular, the obtained changes in behavioral response were indicative of a decrease in the animals' general activity (both motor and learning) and intensification of their emotional discomfort—anxiety in response to unknown surroundings. The males demonstrated quicker adjustment to unknown surroundings, and the females had more pronounced parameters of emotional discomfort.

After 14-day carbacetam administration, changes in the behavior of rats were demonstrated by a reliable decrease of the latent period in representatives of both sexes compared with those rats with metabolic syndrome; the use of GABA-receptor modulator improved the parameters of the general activity of rats. Thus, horizontal motor activity determined by the number of squares crossed by the animals during testing increased by 16.7% in males and 14.1% in females. The index of vertical motor activity estimated by the number of animal stands when they rested on the side walls and when their front limbs were in the air (not rested on the wall) tended to increase. It was 42.5%. The index of the number of examined openings after administration of the medicine increased by 34.5% in males and 14% in females. Analysis of emotional parameters showed that after carbacetam administration the number of washings increased by 38.4% in males and 28.9% in females. Other parameters remained unchanged.

The results indicate different nature of individual adjustment possibilities to the open field conditions in males and females with metabolic syndrome and under the conditions of carbacetam correction. Because today, the scientific community does not have a common opinion concerning the motives determining the behavior of rats in an open field, we can suggest the so-called adjusting behavior of rats. This behavior is formed on the principle of a dynamic central-peripheral organization to achieve a useful adjustment result. On the contrary, achieving results using great efforts can be considered a negative phenomenon.

The results of dynamics in learning and memory of rats with CPAR reproduction are presented in Figure 3 AB. In the control group with CPAR reproduction, 24 hours after learning, 100% of rats remembered electric current stimulation in the dark block and did not enter it. At the same time, the latent period of the first entrance into the dark block in rats with stimulat-

ed pathology decreased reliably, which is indicative of memory impairment. In rats with metabolic syndrome 24 hours after electric stimulation, the latent period of entrance was higher than in the group with provoked pathology before the stimulation. It was 60% higher in males and 29% higher in females, but it was lower than that of the control group by 42% and 45%, respectively.

On the 14th day after carbapenem administration in rats with the pattern of metabolic syndrome and CPAR reproduction, the latent period of entrance into the dark block of the camera decreased by 56.1% in males and by 58.5% – in females compared with the control group. The results obtained inform about memorizing impairment as to the getting a painful stimulus. At the same time, the latent period increased in rats of both sexes after receiving carbacetam in comparison with rats having metabolic syndrome. Thus, the latent period in males increased by 86.9%, and in females – by 84.8%.

Analyzing the results of "open field" and "CPAR" tests, we can suggest that both males and females with metabolic syndrome decrease their motor, locomotor, learning and emotional activity. After administration of a GABA-receptor modulator, motor activity increases, and inconsiderable changes in the emotional behavior of rats by the number of washings occur. The results indicate the experimental rats' behavior modulation due to carbacetam administration. At the same time, effective retention of conditioned passive avoidance reflex to electric painful stimulation in rats receiving carbacetam indicates improved cognitive ability.

Conclusion

Therefore, we have found that metabolic disorders result in reliable changes in all the constituents of cognitive function of the central nervous system in rats of both sexes. These changes include the behavior of rats during their observation in the "open field" (disturbances of motor and learning activity, emotional disorders) and a longer latent period of entrance into the dark block while reproducing conditioned passive avoidance reflexes. At the same time, the study results indicate possible correction of behavioral disturbances of rats of both sexes with metabolic syndrome after carbacetam administration. This is demonstrated by the increased parameters of orientation-learning activity, normalizing the behavioral response of animals, and a longer latent period of entrance into the dark camera block.

Conflict of interest

The authors declare no conflict of interest.

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