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Effects of memantine on the passive avoidance test in young rats

V.A. Gorbachenko, E.A. Lukyanetz

O.O. Bogomoletz Institute of Physiology, NAS of Ukraine, Kyiv; e-mail: elena@biph.kiev.ua

Alzheimer's disease (AD) is a long-lasting progressive neurodegenerative disease that degrades memory and cognitive function and is often complicated by disorientation and other psychiatric syndromes. At present, to improve the condition of patients with AD, for their treatment, use the drug memantine. The drug is a noncompetitive antagonist of NMDA glutamate receptors in the brain. The present experiments aimed to test the influence of memantine on the memory processes in rats. We used the passive avoidance test "Step-down". The latter is used to assess memory function based on the association formed between a particular environment that an animal is learning to avoid and a negative stimulus in the form of a weak electric shock to the feet. We found that memantine significantly, twice, decreased the latency time step-down from the platform in rats during their familiarization with the chamber. The rats became more determined and less afraid of the unknown environment under memantine. Memantine significantly affected the emotionality of young rats, which leads to errors in the passive avoidance test. However, it did not impair memory. It can be concluded that memantine induces a shift toward greater excitability in rats.

Key words: Alzheimer's disease; memantine; rats; behavior; passive avoidance reflex; memory.

INTRODUCTION

It is well known that Alzheimer's disease is a long-lasting progressive neurodegenerative disease that degrades memory and cognitive function and is often get complicated by disorientation and other psychiatric syndromes. It is the most common cause of dementia [1]. Currently, several hypotheses are related to the etiology of this disease. The most known of them are amyloid, acetylcholine, calcium, mitochondrial, and others [2]. In our previous experiments, we have shown the role of mitochondria in amyloid-β action on the nerve and glial cells at modeling AD [3, 4]. We also tested the medicinal preparation memantine, which is used in the treatment of AD to the behavioral responses of rats of different ages (5-7). Also, experiments were carried out that showed the involvement of calcium ions and calcium channels of the neuronal membrane in the mechanisms of action of amyloid-β [8, 9].

At present, several drugs are used for the treatment of AD, and memantine is used to improve the condition of patients with AD among them. Interestingly, memantine is the only FDA-approved drug for treating AD, not an acetylcholinesterase inhibitor [10]. The drug is a non-competitive, low- to medium-affinity antagonist of NMDA glutamate receptors in the brain. Memantine's principal mechanism of action is believed to block Ca^{2+} current flow through pores of NMDA receptor-operated ion channels, reducing the effects of excitotoxic glutamate release. Memantine has a higher affinity than Mg^{2+} ions at the NMDA receptor, thereby blocking prolonged Ca^{2+} influx while preserving transient physiological activation of the ion channels by activity-dependent, synaptically released glutamate [11]. Memantine also is an antagonist of the type 3 serotonin (5-HT3) receptor, and a low-affinity antagonist of the nicotinic acetylcholine receptor, but does not bind other receptors of neurologic or psychiatric drugs, such as adrenergic, benzodiazepine, dopamine, GABA receptors, or voltage-dependent calcium, sodium, or

potassium channels [12]. Therefore, according to the mechanism of its action, the action of memantine can be attributed to preventing of glutamate excitotoxicity and finally, to the calcium hypothesis.

It is known that memory is most affected in AD patients. Therefore, to determine the effectiveness of the action of memantine, it is logical to use specialized behavioral tests for memory and learning in animals. Among other tests, the passive avoidance test "Step-down" [13-16] which is a fear-aggravated test, is used to evaluate learning and memory in rodent models of CNS disorders. In this test, animals learn to avoid an environment in which an aversive stimulus (such as a foot-shock) was previously delivered. This test is widely used to prove the effectiveness of drugs [14] or other influences such as hypoxia etc. [17].

Earlier it was shown that memantine has an individual therapeutic effect on patients. Some of them confirmed its effectiveness, others denied it [18]. Therefore, the present experiments aimed to test the influence of memantine on memory processes in young rats to determine whether memantine really affects memory processes, or its action is directed to other accompanying processes.

METHODS

All experimental procedures followed the European Commission Directive (86/609/EEC) and approved by the local Animal Ethics Committee of the Bogomoletz Institute of Physiology (Kyiv, Ukraine). All efforts were made to minimize the numbers and suffering of animals used. Experiments were carried out on male Wistar rats aged 1–3 months, weighing 80–280 g. Rats were kept under standard vivarium conditions (temperature 23–25°C). Animals were divided into control and experimental groups (n = 5 in each).

Memantine (officinal form Akatinol Memantine) was used. An aqueous memantine solution was administered at a dose of 20 mg/kg per os for 1 h before the experiment.

We used standard protocol, the passive avoidance test "Step-down" (PAT), which, in detail, was described earlier [19]. We used a box for experiments "step-down" (15×25×25 cm) with the raised plastic platform. The passive avoidance test is used to assess memory function based on the association formed between a particular environment that an animal is learning to avoid and a negative stimulus in the form of a weak electric shock to the feet. The essence of the method is that when a rat is placed on a raised platform in the center of a rectangular space, almost immediately, it begins to step-down to the floor to explore the space and approach the walls. The time it takes for the animal to step-down is used to quantify this process. As soon as the animal hits the electrified grid floor, a strong electric shock is given to the feet. The rodent's ability to remember this fact is tested by repeatedly placing the animal back on the platform.

The animal changes its behavior to avoid electrical shock; this manifests in an increase in the delay period or refusal to step-down. Latency is used to evaluate memory. Increasing or decreasing latency allows to get an assessment of the improvement or deterioration of memory and learning processes.

Data are presented as means ± standard error (SE). The statistical significance of differences between the values in control and treated groups was estimated using a Student's t-test; the cases with $P < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

In our experiments, a plastic disk 15 cm in diameter and 5 cm in height was attached in the chamber's center with a grid floor and was movable. The critical moment was placing the animal on the platform. We did not place the rat directly on the platform, since the desire to avoid contact with the experimenter's hand can reduce the latency period of a step-down. Besides, the time for the animal's release from contact with the hands is rather inaccurate; also,

the experimenter's influence can affect the time. To overcome these difficulties, we placed the animal in a glass cylinder covering a plastic platform. A hollow glass cylinder, 30 cm high and 20 cm in diameter was easily slipped onto this round platform. The test rat was placed in a cylinder on a platform (Fig. 1). And the animal was released after 10 s, quickly lifting the cylinder. After raising the cylinder with the animal being on the platform, the latent time countdown began.

The first phase was "familiarization", which consisted of placing the animal on the platform, as described above. Then the step-down latency time (tl) was measured. The latency period was measured from the moment when the animal was step-down on the floor until it removed all four legs from the platform. After that, the animal was placed in a living cage. This procedure was repeated 3 times at 30-minute intervals. It can be seen that tl decreased with each experience as the animal becomes familiar with the new environment. Large initial delays in the descent of the animal from the platform are associated with fluctuations in decision making. During the second and third trials, the mean values of the latent period of descent decreased. The latter is related to the acquired experience of step-down.

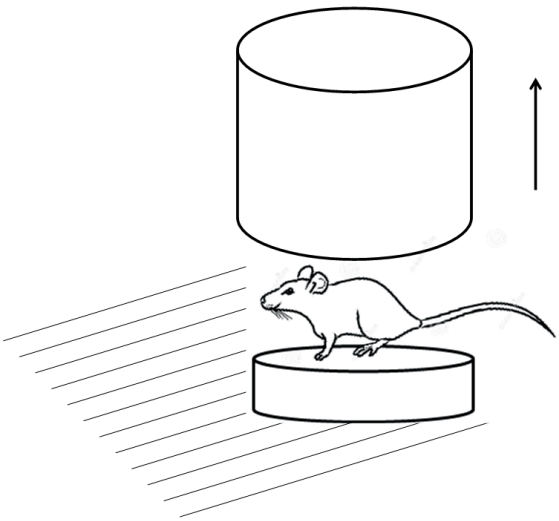

Fig. 1. The platform from which the rat steps down. The cylinder that limits it is also shown. The bar indicates that the cylinder is raised

The experiments showed two groups of rats that differed in the time they left the platform (tl), Fig. 2, A. Some animals performed their descent more quickly, others more slowly. It is known that the behavior of rats has a multidirectional character: active rats have a pronounced orienting-exploratory reaction to an unknown environment, as a result of which they quickly make decisions and are proactive (rats 1, 3, 5 in our case, Fig. 2, A). Animals with a predominance of passive-defensive reaction to new situational conditions (a pronounced vegetative component is noted) make decisions more slowly (rat 2 and 4 on Fig. 2, A). Figure 2, B shows the mean values of the tl, which were 20.88 ± 7.83, 9.05 ± 3.44, and 6.19 ± 2.04 s for 1, 2, and 3 serial trials correspondingly.

In the next experiments, we did the "training" stage. As soon as the rat stepped down from the platform on the third trial, a current (50 Hz, 0.4 mA) was applied to the grid floor for 1 s, and after that, the animals were returned to the living cage. An important point is the timing of the electric current. The current was not supplied at the first contact of the animal with the floor, since a light touch with the front paws does not give the required intensity of the electric shock. Therefore, the current was turned on when the animal stood on the grid floor, with all four paws. The electric shock, used immediately after the third descent, caused a pronounced reaction of fear, characterized by fading, falling to the floor, jumping, puffing up the fur, and heavy breathing. When the rat found a platform, it remained either motionless on it or made studies and jumps in a vertical direction (climbed on its hind legs, sniffed), but avoided descent to the grid floor. In most animals, the latent period of descent exceeded 1 min. Although some animals rushed around, not understanding that they needed to climb onto the platform.

The next step was the "reproduction" stage of the reflex. After 24 h, the rats were placed back on the platform, and the latency of descent was measured. Testing ended when the animal

stepped down or remained on the platform for more than 1 min. The only parameter that was measured was decent latency. These "reproduction" experiments were repeated about once per week. Experiments have shown that the performance of the test by rats is quite individual. A representation of the percentage of successful tests (in %) of all trials for every rat is shown in Fig. 2, C. The figure shows the individual performance of the avoidance test. It can be seen that more successful was rat 1 and more failed rat 2. As can be seen, this test's performance did not correlate with whether the animal belonged to the active or passive phenotype (Fig. 2, A). The shaded columns correspond to the passive phenotype. Figure 2, D presents mean tl values obtained from the control group for seven weeks. As can see, the mean values of tl in the control group, in the beginning, it was small (17.45 ± 4.78 s), then gradually increased, reaching a maximum (57.33 ± 2.67 s) for two weeks, then decreased slightly (46.69 ± 10.94 s). The observed scatter of data was noticeable due to the individual characteristics of animals (Fig. 2, C). In the next experiments, we tested the memantine group. The same experimental protocol was used, and measurements were taken on the same days as the control group. In the first stage, "familiarization", it is proved that the rats are also divided into two groups differing in phenotype, Fig. 3, A. The figure shows that 1st and 2nd rats belonged to the active phenotype and 3, 4, and 5 to a passive one. However, all groups demonstrated a more short time of leaving the platform than the control one. We found that mean values of tl for all three trials were close in meaning and little differed from each other in contrast to the control

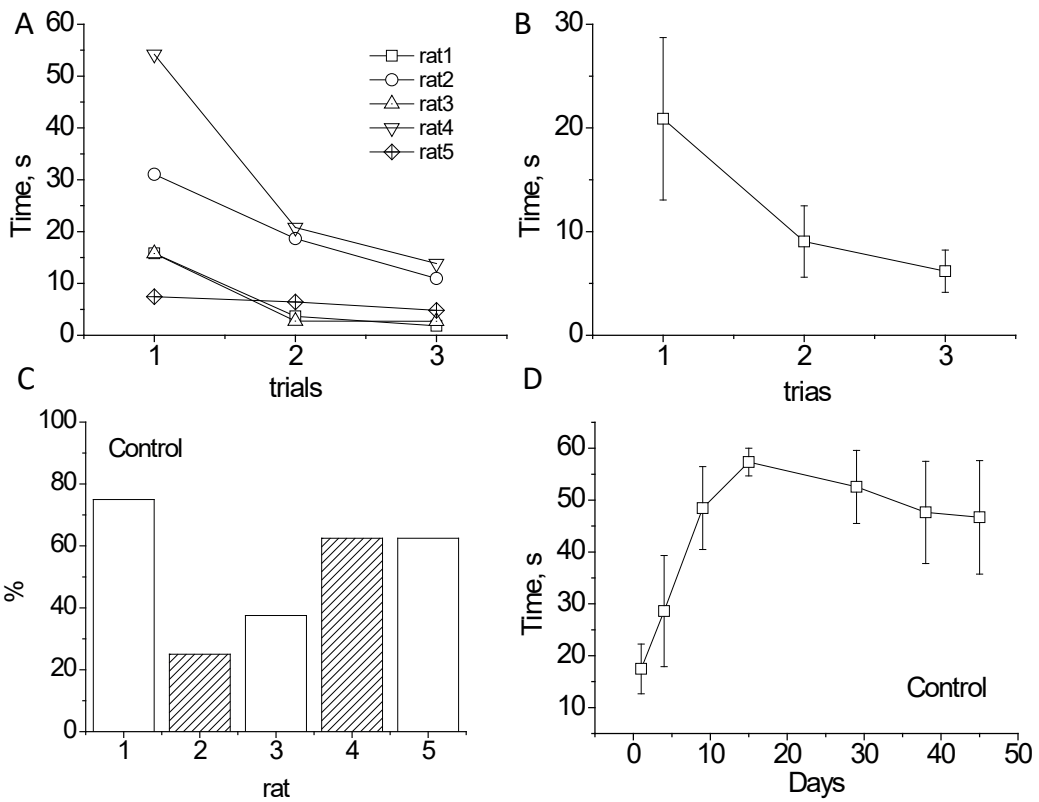

Fig. 2. Reactions of the control group of rats to the passive avoidance test "Step-down". A, the stage of the experiment "familiarization". The latent time of descent (tl) for every trials (1, 2, 3) for every tested rats is presented. B, mean latent time of descent. C, the success of the performance of the test by each rat (stay on a platform more than 1 minute). Columns with a pattern indicate a group with a passive phenotype. C, Changes the mean value of latent time of descent (tl) over a long period

group, in which successive values decreased exponentially (Fig. 2, B). Their values were 9.8 ± 3.61, 8.35 ± 2.63, and 8.5 ± 3.46 s for 1st, 2nd, and 3rd trial correspondingly, Fig. 3, A. It is noticeable that the value of the time of the first attempt is much shorter than in the control group, and it differs more than twice, Fig. 4, A. It can be seen that the mean values of tl are almost the same for all three tests (Fig. 3, B).

In the memantine group, animals were also represented by active and passive (Fig. 3, A). As in the control group, it turned out that the phenotype did not correlate with the animals' success in the passive avoidance test (Fig. 3, C). Figure 3, C shows individual success in performing the test in the rats. It can be seen that the success of individual animals varied significantly; the most successful was rat 3, the less rat 4. However, it should be noted that the latency time for leaving the platform decreased significantly in the entire group (Fig. 3, B). Especially, this concerned the first attempt at the "familiarization" stage. The mean latency time was decreased by two times and accounted for 9.8 s (Fig. 4, A). This phenomenon can be explained by the fact that the rats became more determined and less afraid of the unknown environment under memantine. Average latency values during a prolonged period of measurements are shown in Fig. 3, D. It can be seen that the mean time spent by the rats on the platform increases, reaching an average value for all animals of 50 s, which indicates the consolidation of the passive avoidance reflex in time.

Averaged data on the success of the passive avoidance step-down test is shown in Fig. 4, B. Figure 4, C shows that the success of rats of the memantine group is lower than that of the control. The decrease in latency time in rats treated by memantine can be explained by

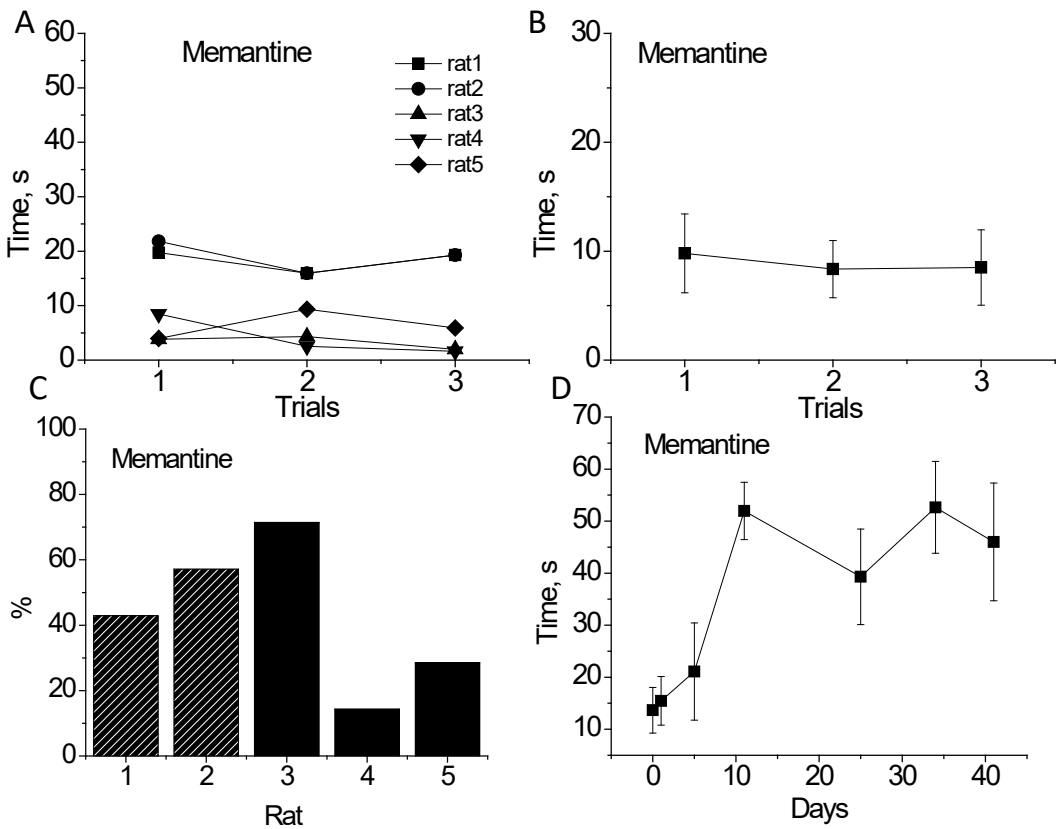

Fig. 3. Reactions of the memantine group of rats to the passive avoidance test "Step-down". The description is the same as in Fig. 2, only with memantine group of rats

the drug's effect on their emotionality. Thus, animals from the memantine group remembered well that one should not go down to the floor from the platform. Still, instead of sitting motionless on the platform, they were extremely active in avoiding being in the experimental chamber. They stood up and constantly jumped to jump out of the experimental chamber. During these jumps, they found themselves outside the platform. As a result, the latent time indicators were recorded and had smaller values. The latter also influenced test success estimation.

It should be noted that the memantine did not significantly affect the changes in the weight gain of the animals (Fig. 4, D), although it tended to slow down weight gain. The latter can be due to the greater emotionality of this group.

Summarizing the obtained data, we can conclude that memantine affects the behavior of young rats. First of all, it influenced rats' emotionality, causing their mobility, searching activity, panic, etc. It was also noticeable that memantine had an individual effect on the passive avoidance test. Some of the rats became very emotional, and in anticipation of an electric shock, they behaved in a panic, trying to jump out of the experimental chamber. In the control group, we did not observe this.

Interestingly, the effect of memantine can be individual and in people. Thus, some patients

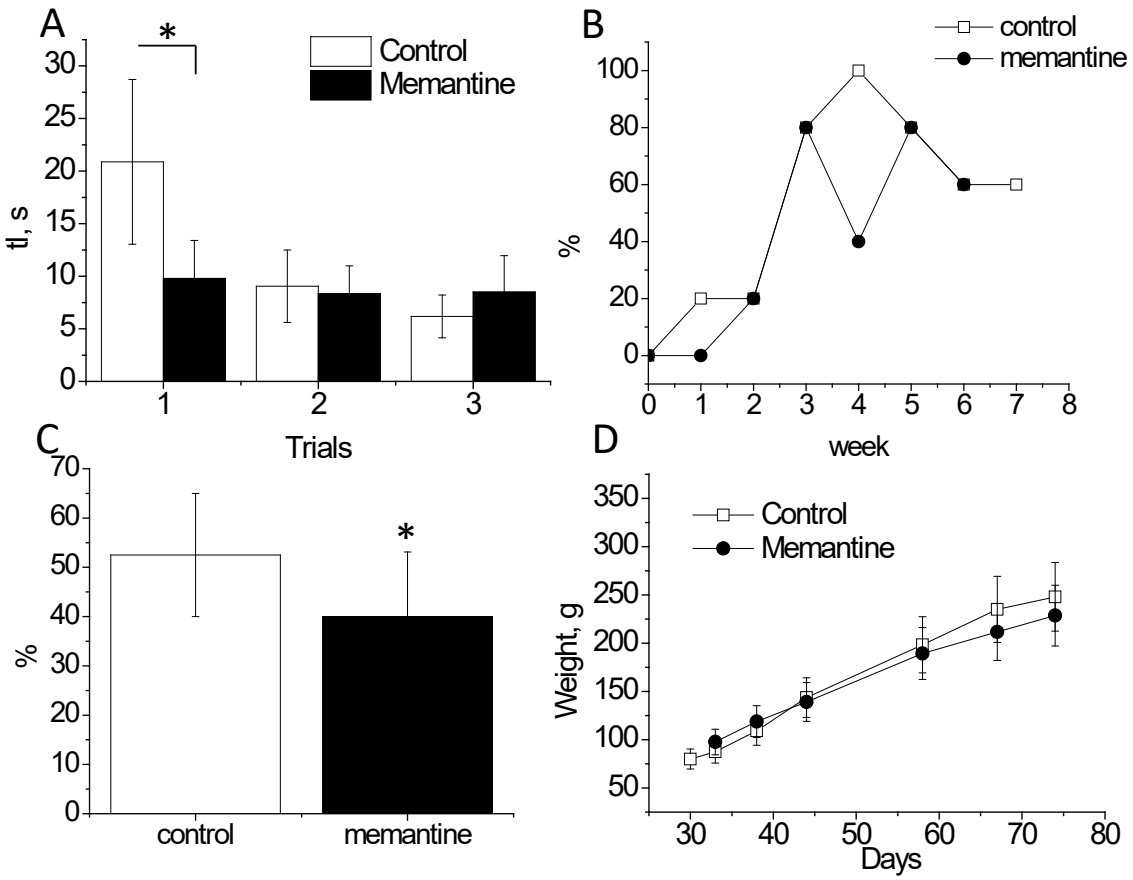

Fig. 4. Comparison of parameters of control and test groups of rats taking memantine. A, the average value of successfully fulfilling the descent avoidance test (stay on platform more 1 min) was recorded for several weeks. B, the mean values of successfully fulfilling the descent avoidance test for all tests are shown. C, mean values of latent time (tl) during "familiarization" stage of the experiment in control and memantine group are shown. D, mean values of changes in body weight of the control and memantine groups are shown. Light symbols – control group, dark –memantine group. Significance of the differences with $P < 0.05$ is indicated by asterisk

noted an improvement in their condition when using memantine, others did not notice the effect [18]. Randomized controlled trials of memantine in AD patients show very different outcomes. With some caution, it may be concluded that possible effects are larger in patients with moderate to severe AD than in patients with mild to moderate AD [20]. Thus, it found the important differences in the efficacy of memantine in mild AD compared to that in moderate-to-severe AD. There is a small clinical benefit of memantine in people with moderate-to-severe AD, which occurs irrespective of whether they are also taking other treatments, but no benefit in people with mild AD [11].

Memantine is one of the few drugs currently approved for the treatment of AD. The clinical effects of memantine are believed to be related to inhibition of the NMDA receptors. Surprisingly, other NMDA receptor blockers have unacceptable side effects that prevent them from being considered for the treatment of AD. One of the mechanisms proposed to explain the therapeutic benefits of memantine involves a preferential reduction in the excitatory impulse to inhibitory neurons in the cortical circuits and subsequent changes in the balance between excitation and inhibition. Research by Povysheva and Johnson elucidated a novel mechanism of action of memantine associated with shifting of this balance away from inhibition in neocortical circuitry [21]. This may explain our results when the memantine group of rats became more emotional, a shift toward greater excitability.

CONCLUSIONS

1. Memantine significantly, twice, decreases the latency time step-down from the platform in rats during their familiarization with the chamber. The rats became more determined and less afraid of the unknown environment under memantine.

2. Memantine significantly affects the emotionality of young rats, which leads to errors in the passive avoidance test. However, it does not impair memory.

3. It can be concluded that memantine induces a shift toward greater excitability in rats.

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В.А. Горбаченко, О.О. Лук'янець

ВПЛИВ МЕМАНТИНУ НА КОГНИТИВНІ ОСОБЛИВОСТІ МОЗКУ МОЛОДИХ ЩУРІВ

Інститут фізіології ім. О.О. Богомольця НАН України, Київ; e-mail: elena@biph.kiev.ua

Метою нашого експерименту була перевірка впливу мемантину на процеси пам'яті у щурів. Ми використовували тест «пасивного уникнення» («Step-down») для оцінки функції пам'яті на основі асоціації, сформованої між конкретним середовищем, яку тварина вчиться уникати, і негативним стимулом у вигляді слабкого електричного удару. Виявлено, що дія мемантину значно зменшує час затримки сходу з платформи у щурів під час їх ознайомлення з камерою (майже вдвічі). Щури ставали більш рішучими і менше боялися невідомого оточення при дії мемантину. Він суттєво впливав на емоційність молодих щурів, що призводило до помилок у тесті «пасивного уникнення». Однак це не погіршувало пам'ять. Можна зробити висновок, що дія мемантину падвищує збудливість у щурів. Ключові слова: хвороба Альцгеймера; мемантин; щури; поведінка; рефлекс пасивного уникнення; пам'ять.

В.А. Горбаченко, Е.А. Лукьянец

ВЛИЯНИЕ МЕМАНТИНА НА КОГНИТИВНЫЕ ОСОБЕННОСТИ МОЗГА МОЛОДЫХ КРЫС

Целью нашего эксперимента было проверить влияние мемантина на процессы памяти у крыс. Мы использовали тест «пассивного избегания» («Step-down») для оценки функции памяти на основе ассоциации, сформированной между конкретной средой, которую животное учится избегать, и негативным стимулом в виде слабого электрического удара. Обнаружено, что действие мемантина зна-

чительно уменьшает время задержки схода с платформы у крыс во время их ознакомления с камерой (в два раза). Животные становились более решительными и меньше боялись неизвестного окружения. Он существенно влиял на эмоциональность молодых крыс, что приводило к ошибкам в тесте «пассивного избегания». Однако это не ухудшало память. Можно сделать вывод, что действие мемантина повышает возбудимость у крыс.
Ключевые слова: болезнь Альцгеймера; мемантин; крысы; поведение; рефлекс пассивного избегания; память.

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