



FILGRASTIM IMPROVED SPATIAL MEMORY FUNCTIONS IN RAT MODEL OF SCOPOLAMINE INDUCED ALZHEIMER TYPE MEMORY DYSFUNCTION

Hamid Sepehri^{1*} and Hadi Nasiri²

¹Neuroscience Research Center, Golestan University of Medical Sciences, Gorgan, Iran.

²Medical Student, Faculty of Medicine, Department of Physiology, Golestan University of Medical Sciences, Gorgan, Iran

Filgrastim is a granulocyte colony-stimulating factor (G-CSF), which also showed effects on memory and cholinergic activity. This study was conducted to determine the effect of filgrastim on memory in Rat Model of scopolamine induced memory dysfunction.

Materials and Methods: 24 male Wistar rats were divided into four groups: Group I: control rats receiving normal saline. Group II: rats induced Alzheimer disease by injection of Scopolamine. Groups III and IV: AD rats treated with 50 and 70 µg/kg/day intraperitoneally (i.p) filgrastim over a period of two weeks. Then, rats trained with four trials per day for 5 consecutive days in the Morris water maze (MWM) to find a hidden platform. Time elapsed for finding the hidden platform were considered as criteria for learning. On the 6th day, memory retention was evaluated by time spent in target quadrant. **Results:** In Scopolamine groups, escape latency during training trials showed a significant decrease ($P < 0.001$) and these rats spent shorter time in the target quadrant in probe trials compared to controls ($P < 0.001$). treatment of scopolamine group by filgrastim in doses of 50 and 70 µg/kg/day significantly reduced the latency time to finding the escape platform ($P < 0.01$). And in probe trials, on the last day of training, the filgrastim –treated group spent significantly longer time in the platform quadrant when compared with the scopolamine induced AD animals ($P < 0.01$). **Conclusion:** filgrastim acted as a memory enhancer in scopolamine induced Alzheimer rats. This cognitive enhancer effect of filgrastim may be attributed to its cholinergic effect.

INTRODUCTION

Alzheimer's is the most common type of dementia which characterized by the accumulation of amyloid plaques, hyper phosphorylation of tau protein, oxidative stress, neuroinflammation leading to neuronal death¹. The hippocampus cholinergic deficit is strongly associated Alzheimer's^{2&3}. Therefore one of the most common treatments of AD is administrating acetylcholine esterase inhibitors⁴. However, these treatment may produce peripheral cholinergic side effects that may diminish their use⁵. Therefore, researches have been directed towards the use of other anti-amnesic therapies with different mechanism with lower side effects.

Granulocyte colony stimulating factor (G-CSF; filgrastim) is a growth factor cytokine that stimulates the proliferation, and maturation of myeloid progenitors in the bone marrow. G-CSF is using for recovery of patients from neutropenia after cytotoxic therapy⁶. The receptor (G-CSF-R) also been reported to be expressed in neural progenitor cells and its expression is induced by different pathogenic stimulus⁷. Numerous reports have described the efficacy of G-CSF on cognitive enhancement in Alzheimer disease model. G-CSF has also been shown to have beneficial effects in the brain trauma and stroke⁸. G-CSF goes through the intact blood-brain barrier and allowing peripheral neuroprotective properties through interactions with G-CSF receptors^{9,10}. For the neuroprotective properties of G-CSF, several

mechanisms including stimulation of neurogenesis and synaptogenesis¹¹⁻¹², angiogenesis¹³, Attenuating of apoptosis and inflammation¹⁴, has been proposed. In our previous study we did not observe any changes in the gene expression of ChAT and AChE in the hippocampus¹⁵. Our study was on healthy rats, and maybe in cholinergic deficit. We could see the beneficial effects of filgrastim. Scopolamine is an anti-muscarinic that by inhibition of acetylcholine neurotransmission induced dementia which is used in an animal model for investigating Alzheimer diseases¹⁶. The present study was designed to investigate the possible ameliorative effect of G-CSF treatment on scopolamine-induced dementia using the water maze.

MATERIAL AND METHOD

Animals and Treatments

24 male Wistar rats weighing 120-150 g were used throughout the experiment. They were obtained and treated in the Golestan animal house. Rats were randomly allocated into 4 groups (6 rats/group in the object recognition test) as follows: Group I received saline (0.9% NaCl solution) and served as control while group II received scopolamine (16 mg/kg, i.p.)²⁴ and served as Alzheimer model. Both groups received saline for 14 days. Groups III-V rats received filgrastim (50, 70 µg/kg/day), respectively. Filgrastim was purchased from Ariatinagen Company (Gorgan, Iran), and after dissolving in saline, administered intraperitoneal for two weeks. Scopolamine (SigmaAldrich, Steinheim, Germany) was administered as a single dose two days before beginning treatment in groups (II-III-V). The behavioral test started 2 weeks after the scopolamine injection. The study was performed according to the guidelines for laboratory animal use and care set by the Animal Ethics Committee of Golestan University of Medical Sciences (GOUMS). With ethical code (ir.goums.rec.1395.41). The animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22±2 °C) and humidity (55±5%) with 12:12 h light and dark cycle.

Morris water maze (MWM) experiment

The MWM used a contained of a black circular pool (160 cm diameter, and 60 cm high) filled with water (30 cm depth) with a temperature of 24±2 °C¹⁷. The pool was separated into 4 quadrants randomly labeled northeast, northwest, southeast, and southwest. A submerged plexiglass platform (10 cm × 10 cm) was hidden 1 cm below the water surface and fixed in the center of the northwest quadrant. The animals received four sessions of training with the hidden platform for five days, with 60s intersession intervals two weeks after scopolamine injection. Each session started by putting the rat at one of 3 start points while facing the wall of the pool. The start location was changed in each training session. The training session was finished when the animal entered the platform. The rat was replaced on the platform for 15s if it was unable to find the platform within the 60s. In the acquisition of the spatial navigation task, the groups performed 1 session of 4 trials each day (day 1-5; trial 1-20). Spatial memory was assessed in a probe trial on the sixth day (trial 21). The platform was removed and rats were permitted to swim for the 60s. A computerized video tracking system (Maze router, Urmia Instruments Inc) was used to monitor the path of the animals in the maze. Parameters including the latency (the time it takes to reach the platform), swimming speed in the training trials were measured. And during the trial probe, the percentage run time in the quadrant of the water bath where the concealed platform had been located in the training trials were measured.

Inhibitory Avoidance Apparatus (Shuttle box)

The passive avoidance test was started 2 weeks after the scopolamine injection using a step-through inhibitory avoidance apparatus. It consisted of two boxes of the same size (20 × 20 × 30 cm). There was a guillotine door in the middle of a dividing wall. The walls and floor of one compartment consisted of white opaque resin and the other one was dark. Intermittent electric shocks (50 Hz, 3 seconds, 1.5 mA intensity) were delivered to the grid floor of the dark compartment by an isolated stimulator. Each animal was gently placed in the white compartment and after 5 seconds the guillotine door was opened and the animal was allowed to enter the dark module (11). Once the animal

entered with all four paws to the next chamber, the guillotine door was closed and the rat was immediately withdrawn from the compartment. This trial was repeated after 2 minutes. As in the acquisition trial, when the animal entered the dark (shock) compartment the door was closed, and a foot shock (50 Hz, 1.5 mA, 3 seconds) was immediately delivered to the grid floor of the dark room. After 20 seconds, the rat was removed from the apparatus and placed temporarily into its home cage. Two minutes later, the animal was retested in the same way as in the previous trials; if the rat did not enter the dark compartment during 300 seconds, a successful acquisition of inhibitory avoidance response was recorded. Otherwise, when the rat entered the dark compartment (before 300 seconds) a second time, the door was closed and the animal received the shock again. Twenty-four hours later, each rat was again placed in the light chamber (retention trial) and after.

Statistical analysis

The results are presented as Mean±S.E.M and analyzed using SPSS 17.0. All of the data were analyzed by the one-way analysis of variance (ANOVA) and Student's T test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results

Figure 1 shows the time taken to reach the hidden platform (A) on each training day for Scopolamine induced Alzheimer Disease (AD) and Control group. The mean escape latency for the trained rats was decreased over the course of the 20 learning trials in both groups. Control group rats (vehicle treated) exhibited significantly lower escape latency in all days during training trials compared to AD group ($P < 0.01$). Two-way repeated measurement of ANOVA test revealed that Scopolamine model significantly increased escape latency compared to control group rats on day 1, 2, 3, 4, and 5 ($P < 0.001$).

Figure 2 shows the time taken to reach the hidden platform on each training day among the filgrastim treated and scopolamine induced AD groups. Morris water maze performance task data indicated that filgrastim in both doses (50 and 70 $\mu\text{g}/\text{kg}$) significantly attenuate escape latency ($P < 0.001$) in AD model of rats compared to scopolamine group on day 1, 2, 3,

4, and 5. No significant differences were observed among the quercetin-treated groups. For all the days the scopolamine treated animals shows a significant difference in the escape latency, suggesting that filgrastim groups performed significantly better than controls over time.

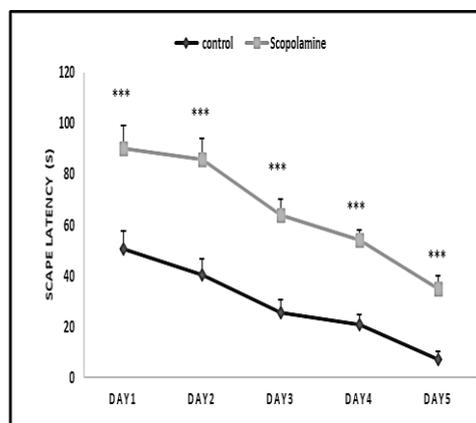


Fig. 1: Comparison of escape latency during training trial days. The figures show the escape latency significantly increased in AD model group rats at the 1th, 2th, 3th, 4th and 5th days of training compared to the control group (** $p < 0.01$, *** $p < 0.001$, respectively). Data express as mean \pm SEM of 6 rats in each groups.

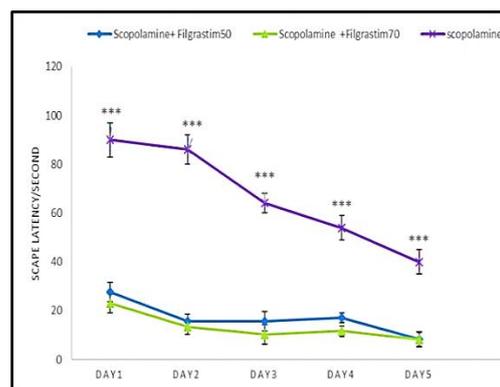


Fig. 2 : Comparison of escape latency during training days. Values are mean \pm SEM. $n=6$ in each groups. *** $P < 0.001$ in two-way repeat measure ANOVA.

Swimming speed during the acquisition test for representative animals in each group are shown in Figure 3. There were not any significant difference changes among the four groups from the mean swimming speed between days during the study, indicating that all of the groups have similar motor capabilities. From this we assume that the rats were able to swim acceptably.

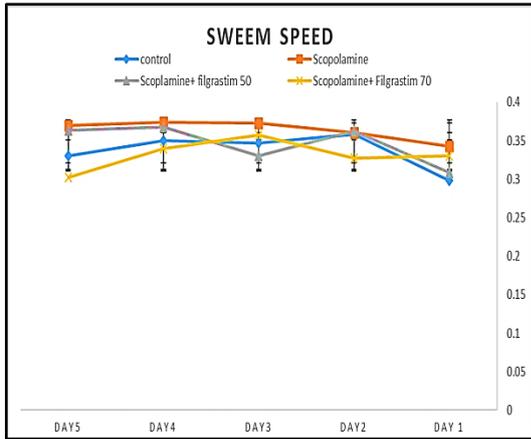


Fig. 3: Comparison of swimming speed during training days. Values are mean ± SEM. n= 6 in each groups. There were no significant differences in swimming speed between different groups

The probe trial data of the Morris water maze study on the last day (6th) shows that the average time spent in target quadrant, where the hidden platform was previously placed, was significantly increased with Filgrastim treatment compared to scopolamine group ($P < 0.01$) (Fig. 4). It was further observed that the scopolamine group significantly impaired the memory retention and the target quadrant preference was diminished significantly in Alzheimer animals model. The treatment with Filgrastim in both doses significantly prevented the memory impairment as indicated by the increase in the time spent in target quadrant ($P < 0.01$). Data from probe trial test indicate that Filgrastim treatment significantly improves the memory deficits seen in AD model rats and both Filgrastim treated groups showed a clear preference for the former platform quadrant (ANOVA, $p < 0.01$). There were no significant differences in time spent in the platform quadrant between the filgrastim groups.

Passive avoidance test in rats

The latency was measured in pre foot shock (Acquisition time) and 24 hrs. post foot shocks (Retention time). Results indicated that the pre-shock latency was the same among all groups, (data not shown). However, the retention time during testing (i.e., testing latency carried out 24 hrs. after receiving foot shock) was significantly decreased in group Scopolamine when compared to other groups

(Co, Alz- Filgrastim50 , Alz and Filgrastim 70; $P < 0.001$, $P < 0.01$, $P < 0.01$, respectively).. Also, there were not significant differences in retention time between control group, as well as Alz and filgrastim(Figure 5).

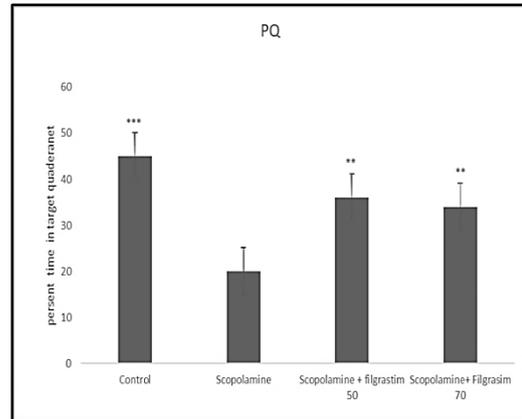


Fig. 4: present time spent in the target quadrant against the average time spent in three other quadrants in probe trial on 6th day Values are mean±SEM. Asterisks indicate a significant difference from the control group (** $p < 0.01$, *** $p < 0.001$)

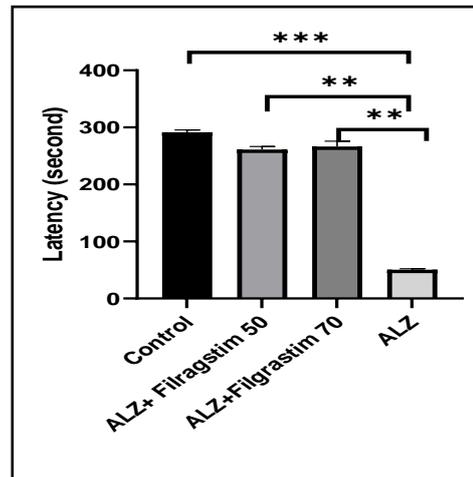


Fig. 5: Comparison of latency to enter the dark chamber 24 hrs. after receiving foot shock (the retention time). Each bar represents the mean ± S.E.M. The retention time was significantly decreased in the Alz group compared to others (Alz _ Filgrastim 50 and Filgrastim 70 and *** $P < 0.001$, ** $P < 0.01$, ** $P < 0.01$ respectively). All results were analyzed by Kruskal-Wallis test followed by Mann-Whitney U test.

Discussion

Our results showed that scopolamine deteriorates spatial and passive avoidance memory. We found that the escape latency and swim path to find a hidden platform in MWM significantly increased in all 5 days' trials in scopolamine treated rats and time spent in the platform quadrant significantly decreased at the sixth day in scopolamine treated rats, in addition the passive avoidance retention and retrieval function was impaired which confirmed the scopolamine -induced memory deficits.

Scopolamine is a anti muscarinic that by inhibition acetylcholine neurotransmission induced memory dysfunction which is used in animal model for investigating Alzheimer diseases¹⁶

Filgrastim treatment with two doses caused a significant decrease in escape latency during training days compared to control group. Also, filgrastim treated animals found the hidden platform sooner than scopolamin group in probe trial. These data provide evidences that filgrastim improved spatial learning and memory capabilities both in acquisition and retention in Morris water maze performance task. Previous report has shown that filgrastim treatment (5-20 µg/kg, p.o., , 40 days) in aged rats rats improved spatial memory in Morris water maze test¹⁸. In a similar study, Sikoglu et al. observed the immediate effects of Granulocyte colony stimulating factor (G-CSF) in spatial memory recovery in a rat model of traumatic brain injury¹⁹. Improvement of memory by filgrastim in antimuscarin induced dementia indicate role of cholinergic system. Previous study showed that GCSF was able to up-regulate nicotinic AChR in the brain of Alzheimer's disease model mice. They suggested that nAChR is essential for inhibiting inflammatory cytokine synthesis by the cholinergic anti-inflammatory pathway²⁰. Other study also demonstrate that G-CSF, augment choline acetyltransferase activity in primary cultured neurons and in cholinergic hybridoma cell line²¹.while in our previously we didn't observed changes in gen expression of ChAT and AChE in frontal cortex and hippocampus after treatment by filgrastim¹⁵. The precise reason for this discrepancy probably was due of work on normal rats but in the Present study we examined the beneficial effect of filgrastim in destroyed cholinergic system. In the present study filgrastim acted as a memory enhancer in

scopolamine induced Alzheimer rats. This cognitive enhancer effect of filgrastim may be attributed to its cholinergic effect and can be useful in Alzheimer diseases .

Acknowledgement

The authors wish to thank all who contributed to this study. This research has been financially supported by the Research Affairs division of Golestan University of Medical Sciences Gorgan, Iran.

REFERENCES

1. H. Duan J. Jiang, J. Xu, H. Zhou, Z.Huang and Z.Yu, "Differences in A β brain networks in Alzheimer's disease and healthy controls", *Brain Res*, 1655, 77-89 (2017).
2. E. K. Perry, M. Curtis, D. J. Dick, J. M. Candy and J. R. Atack, "Cholinergic correlates of cognitive impairment in Parkinson's disease: comparisons with Alzheimer's disease", *J Neurol Neurosurg Psychiatry*, 48, 413-421 (1985).
3. Y. Oda, "Choline acetyltransferase: The structure, distribution and pathologic changes in the central nervous system", *ResearchGate*, 49, 921-937 (1999).
4. D. K. Lahiri, J. T. Rogers, N. H. Greig and K. Sambamurti, "Rationale for the Development of Cholinesterase Inhibitors as Anti- Alzheimer Agents", *Curr Pharm Des*, 10, 3111-3119 (2004).
5. J. Godyń, J. Jończyk, D.Panek and B. Malawska, "Therapeutic strategies for Alzheimer's disease in clinical trials", *Pharmacol Rep*, 68, 127-138 (2016).
6. B. N. ervi, D. C. Link and J. F. DiPersio, "Cytokines and hematopoietic stem cell mobilization", *J Cell Biochem*, 99, 690-705 (2006).
7. F. Kirsch, C. Krüger and A. Schneider, "The receptor for Granulocyte-colony stimulating factor (G-CSF) is expressed in radial glia during development of the nervous system", *BMC Dev Biol*, 8 (1), 1-7 (2008)
8. W. R. Schäbitz, R. Kollmar, M. Schwaninger, E. Juettler and J. Bardutzky, "Neuroprotective effect of granulocyte colony-stimulating factor after focal

- cerebral ischemia", *Stroke*, 34, 745-751 (2003).
9. A. K.Schmidt, A. Reich, B. Falkenburger, J. B .Schulz, L.O. Brandenburg and S. Ribes, "Tauber Adjuvant granulocyte colony-stimulating factor therapy results in improved spatial learning and stimulates hippocampal neurogenesis in a mouse model of pneumococcal meningitis", *J Neuropathol Exp Neurol*, 74, 85-94 (2015).
 10. Y. N. Yang, C. Lin, C. Yang, Y. Lai, P. Wu and S. N. Yang, "Neurogenesis recovery induced by granulocyte-colony stimulating factor in neonatal rat brain after perinatal hypoxia", *Pediatr Neonatol*, 54, 380-388 (2013).
 11. M. Komine-Kobayashi, N. Zhang, M.Liu, R.Tanaka, H. Hara and Akimichi Osaka, "Neuroprotective effect of recombinant human granulocyte colony-stimulating factor in transient focal ischemia of mice", *J Cereb Blood Flow Metab*, 26, 402-413 (2006).
 12. J. Sanchez-Ramos "Pilot study of granulocyte-colony stimulating factor for treatment of Alzheimer's disease", *J Alzheimers Dis JAD*, 31, 843-855 (2012).
 13. H. H, Z.Qi, L.Jiejie, H.Haojie, Granulocyte-Colony Stimulating Factor (G-CSF) Accelerates Wound Healing in Hemorrhagic Shock Rats by Enhancing Angiogenesis and Attenuating Apoptosis. *Medical science monitor basic research*, 23, 2644-2653 (2017).
 14. K. Kamezaki, C. Cimino, R. Avila, A. Rowe, R. Chen and G. Whelan "The Roles of Stat3 and ERK in G-CSF Signaling", *Blood*, 104, 2172-2172 (2004).
 15. A. Parhizkar, F. Ganji, Z. Nazari and H.Sepehri, "Effects of filgrastim on gene expression of cholinergic system in the rat hippocampus", *Natl J Physiol Pharm Pharmacol*, 7(5), 513-516 (2017).
 16. N.M. Navarro, M.C. Krawczyk, M.M. Boccia and M.G. Blake "Extinction and recovery of an avoidance memory impaired by scopolamine", *Behav Pharmacol*, 4,249-254 (2013).
 17. C. V. Vorhees, M. T. Williams, "Morris water maze: procedures for assessing spatial and related forms of learning and memory", *Nat. Protoc.*, 1, 848-858 (2006).
 18. H. Sepehri and E. Mehrazin, "Effect of filgrastim (recombinant human granulocyte colony stimulating factor) on spatial memory in aged rats", *Natl J Physiol Pharm Pharmacol*, 5, 421-425 (2015).
 19. E. M. Sikoglu, M. E. Heffernan and K.T. Kenneth, "Enhancement in cognitive function recovery by granulocyte-colony stimulating factor in a rodent model of traumatic brain injury", *Behav Brain Res*, 259, 354-356 (2014).
 20. H. Jiang, C. X. Liu, J. B. Feng, P. Wang, C. P. Zhao, Z. H. Xie, Y. Wang, S.L. Xu, C, Y Zheng and J. Z. Bi, "Granulocyte colony-stimulating factor attenuates chronic neuroinflammation in the brain of amyloid precursor protein transgenic mice: an Alzheimer's disease mouse model", *J Int Med Res*, 38, 1305-1312 (2010).
 21. Y. Konishi, D. H. Chui, H. Hirose, T. Kunishita and T. Tabira, "Trophic effect of erythropoietin and other hematopoietic factors on central cholinergic neurons in vitro and in vivo", *Brain Res*, 609(1-2), 29-35 (1993).



نشرة العلوم الصيدلانية جامعة أسيوط



تحسن وظائف الذاكرة المكانية في نموذج الفئران جرذان لخلل الذاكرة من نوع الزهايمر المستحث بالسكوبولامين الناتج عن الفيلجراستيم

حميد سبهي^١ - هادي نصيري^٢

^١مركز أبحاث علم الأعصاب ، جامعة جولستان للعلوم الطبية ، جرجان ، إيران.

^٢طالب طب ، كلية الطب ، قسم علم وظائف الأعضاء ، جامعة جولستان للعلوم الطبية ، جرجان ، إيران

الفيلجراستيم هو عامل تحفيز مستعمرة المحبيبات (G-CSF) ، والذي أظهر أيضاً تأثيرات على الذاكرة والنشاط الكوليني. أجريت هذه الدراسة لتحديد تأثير الفيلجراستيم على الذاكرة في نموذج الجرذان لضعف الذاكرة الناجم عن السكوبولامين.

المواد والطرق: تم تقسيم ٢٤ ذكور من جرذان ويستار إلى أربع مجموعات: المجموعة الأولى: الفئران الضابطة التي تتلقى المحلول الملحي الطبيعي. المجموعة الثانية: الجرذان المستحثة بمرض الزهايمر عن طريق الحقن بمجموعتي سكوبولامين. المجموعتين الثالثة والرابعة: عولجت الجرذان بمقادير ٥٠ و ٧٠ ميكروجرام / كجم / يوم فيلجراستيم داخل الصفاق (i.p) على مدى أسبوعين. بعد ذلك ، تم تدريب الجرذان بأربع تجارب يومياً لمدة ٥ أيام متتالية في مناهة موريس المائية (MWM) للعثور على منصة مخفية. تم اعتبار الوقت المنقضي للعثور على النظام الأساسي المخفي كمعيار للتعلم. في اليوم السادس ، تم تقييم الاحتفاظ بالذاكرة من خلال الوقت الذي تم قضاؤه في الربع المستهدف.

النتائج: في مجموعات سكوبولامين ، أظهر كمون الهروب أثناء التجارب التدريبية انخفاضاً كبيراً (P < 0.001) وقضت هذه الجرذان وقتاً أقصر في الربع المستهدف في تجارب المسبار مقارنة بالمجموعات الضابطة (P < 0.001). خفضت معالجة مجموعة سكوبولامين بواسطة الفيلجراستيم بجرعات ٥٠ و ٧٠ ميكروجرام / كجم / يوم بشكل كبير من وقت الكمون لإيجاد منصة الهروب (P < 0.001). وفي تجارب المسبار ، في اليوم الأخير من التدريب ، أمضت المجموعة المعالجة بالفيلجراستيم وقتاً أطول بشكل ملحوظ في ربع المنصة بالمقارنة مع حيوانات الزهايمر المستحث بالسكوبولامين (P < 0.001).

الخلاصة: فيلجراستيم كان بمثابة محسن للذاكرة في جرذان الزهايمر المستحث بالسكوبولامين. يمكن أن يعزى تأثير المحسن المعرفي لـ الفيلجراستيم إلى تأثيره الكوليني.