# INFLUENCE OF GALANTAMINE/4-AMINOPYRIDINE COMBINATIONS ON ACTIVE AVOIDANCE AND LOCOMOTOR ACTIVITY IN MICE

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**Abstract.** Alzheimer's disease (AD) is a progressive neurodegenerative disease affecting learning and memory processes. The most commonly used drugs for symptomatic treatment of AD are the inhibitors of the enzyme acetylcholinesterase (AChE) – Galantamine, Rivastigmine, Donepezil. The aim of this study is to investigate the effects of the combination galantamine/4-amynopyridine (Gal/4-AP) in different ratios on locomotor activity after single and on active avoidance after repeated administration in mice. Galantamine and 4-aminopyridine alone, as well as their combinations didn't influence significantly locomotor activity in mice at doses representing 1/20 of $LD_{50}$ of the respective substances. The memory enhancing potential of the compounds was examined using the active avoidance test and for deterioration of memory processes was used scopolamine in dose 3 mg/kg. The results demonstrate that among the Gal/4-AP combination in ratios 1:1, 2:1 and 3:1, the highest effect was observed in the group, treated with 3:1 ratio. In this group there is a significant increase in the number of responses avoided and escaped in active avoidance test.

**Key words:** scopolamine, galantamine/4-aminopyridine combinations, locomotor activity, active avoidance

## Introduction

Alzheimer's disease (AD) is the leading cause of dementia, a general term of memory loss and disturbances in other abilities (language, orientation, emotions) serious enough to interfere with daily life. AD accounts for 60 to 80 percent of all dementia cases [1].

The risk for development of AD in the elderly is mainly related to complex interactions between biological factors, environmental impacts throughout life and family history. Age is the main known risk factor for AD, with the risk being doubled every 5 years after the age of 65 [2].

AD is characterized by progressive memory decline with impairment of at least one other cognitive function . In AD the major brain areas affected are the cortex and limbic system. The disease often begins with symptoms like short-term memory loss and continues with more widespread cognitive and emotional dysfunctions. The loss of cholinergic neurons in the hippocampus and frontal cortex is a pathogenic mechanism of the disease and is thought to underlie the cognitive deficit and loss of short-term memory that occurs in AD [3].

The discovery of cholinergic hypothesis by White and colleagues in 1976 [4], leads to the application of different therapeutic approaches aimed to enhance cholinergic transmission [5]. Currently, the cholinesterase inhibitors – donepezil, rivastigmine, galantamine and N-methyl-D-aspartate receptor antagonst memantine are the currently used drugs for symptomatic treatment of AD. Beside acetylcholinesterase inhibitors, acetylcholine (Ach) enhancement is obtained by modulating both ligand and voltage-gated ($Ca^{++}$ and $K^{+}$) ion channels. Agents with later activity, such as the potassium channel modulator 4-aminopyridine (4-AP) have been clinically evaluated as Ach release enhancing agents [6]. The aim of this paper is to establish the effect of combination of galantamine and 4-aminopyridine in different ratios on scopolamine-induced memory impairment in mice using locomotor activity and active avoidance tests.

## Materials and Methods

### *Animals*

Experiments were performed on male albino mice line H, 28-32 g body weight (b.w.), supplied by Experimental and breeding base for laboratory animals – Slivnitza, Bulgaria. Mice were housed by groups in standard polycarbonate cages; water and food were available ad libitum, room temperature 22±3ºC, humidity 30%, lighting schedule 12 h light/dark cycle. Animals were trained and tested during light part of the cycle. All experiments were approved by the Institutional Animal Care Comitee at the Medical University of Sofia, Bulgaria.

### *Locomotor activity*

Locomotor activity was assessed in plexiglas activity cage – Ugo Basile Biological Research Apparatus, Milan, Italy (38 cm long, 25 cm wide, 23 cm high) that was fitted with grid bars running parallel to each other. When crossing these bars mice activate a circuit which transforms these movements into signals that can be registered as count per a certain time. Groups of 6 male mice each were placed in the activity cage 5 min after the intraperitoneal injection of 1/20 of LD50 of the respective compound. The mobility of each group was measured for 1 hour and the total number of counts during each 10-min period was recorded. The effect of each compound on locomotor activity was expressed as counts/10 min for 1 hour [7, 8].

### *Active avoidance after repeated administration of compounds*

The experiments were carried out on 42 male mice, line H, 28-32 g b.w, divided into 7 groups (n=6). The training degree and dynamics were examined by the method of step-through active avoidance test with an automated shuttle-box (Gemini Avoidance System, San Diego, CA, USA), divided into two identical compartments of the same size (25 cm long x 20 cm wide x 16 cm high) separated by a door (5 cm wide x 6 cm high). Briefly, in active avoidance testing, after acclimatization period in the chamber in which was the animal the light was automatically turned off and 5 seconds later a electrical foot shock was applied through the grid floor. The animal must than leave the dark site of the test station and enter into the lighted chamber to escape the foot shock. The animal is then returned to their home cage. After the training day each animal was trained for four consecutive days (day 1 to day 4) with a series of 20 stimuli including conditioned (light of 15 W, sound of 3000 Hz, 50%) and unconditioned stimuli (foot shock – 1mA, 3s). The tested compounds were administered intraperitoneally (i.p.) before the active avoidance test from day 1 to day 4 and after that every day for 8 days without testing the animals in the apparatus. Memory impairment was induced by treatment with scopolamine (3 mg/kg, i.p.) 30 minutes after administration of compounds, and the active avoidance test was performed 30 min after treatment with scopolamine. Scopolamine was administered on day 1, day 4 and day 12. After 4 days of training the memory storage processes were studied 8 days later again by active avoidance test in GEMINI apparatus [9].

GEMINI software automatically records

three different outcomes: "avoided", when the subject takes its cue from the conditioned stimulus (light and sound) and leaves before the administration of the unconditioned stimulus (foot shock); "escaped", when the subject leaves during the administration of the unconditioned stimulus and "no response", when the subject didn't leave the dark chamber during conditioned and unconditioned stimuli.

***Drug administration***

The solutions were prepared *ex tempore* with saline and were introduced intraperitoneally in a volume of 0,1 ml/10g body weight. Scopolamine (Sigma, India) was administered at dose 3 mg/kg. The combination galantamine/4-AP (Gal/4-AP) in different weight/weight (*w/w*) proportions, galantamine (Sopharma, Bulgaria) and 4-AP (Sigma - Aldrich, USA) were administered at dose 1/20 of LD50 of the respective compound. The control group of animals received the dissolver (sodium chloride 0.9%) in the same volume. Drug treatment groups: control group – sodium chloride; scopolamine – 3 mg/kg; 4-AP – 0,3 mg/kg; galantamine – 0,75 mg/kg; 1:1 – 0,6 mg/kg; 2:1 – 0,6 mg/kg ; 3:1 – 0,6 mg/kg.

**Statistical analysis**

The statistical analysis was performed by Student's (paired) t-test. In the analysis of differences between groups, values of $p \leq 0,05$ and $p \leq 0,01$ were considered statistically significant.

**Results**

**Effect on spontaneous locomotor activity.** The locomotor activity of combination Gal/4-AP in different ratios was recorded 5 min after i.p. administration of 1/20 part of LD50. In all groups, except control group, there is no significant change observed in locomotor activity (Figure 1).

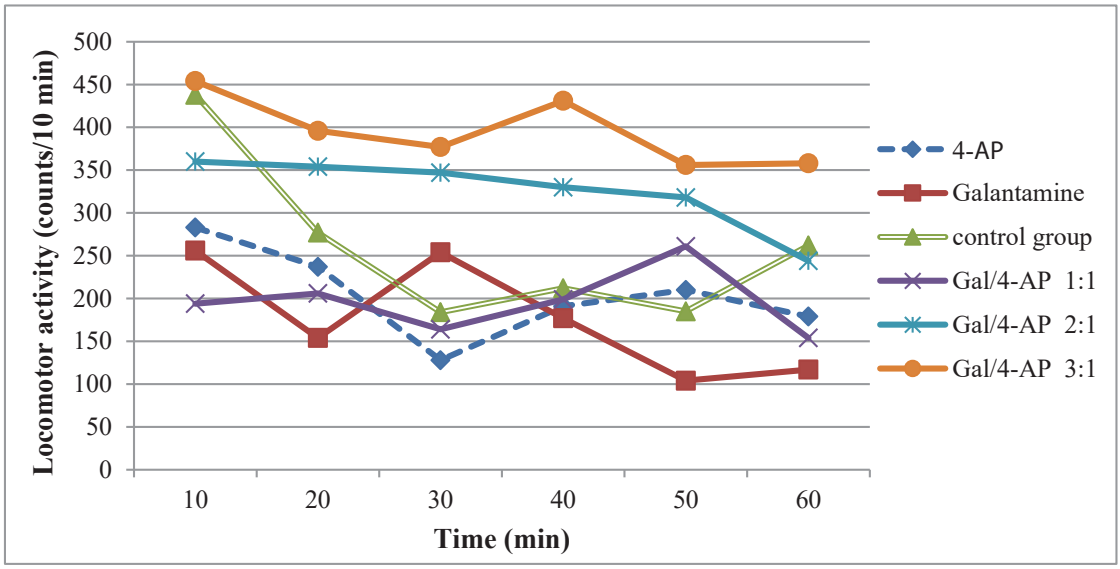


**Fig.1.** Effect of galantamine, 4-AP and different combinations on spontaneous locomotor activity after i.p. administration of 1/20 of LD50. The effect of each compound on locomotor activity was expressed as counts/10 min for 1 hour.

After repeated administration of combination Gal/4-AP in different ratios in all groups was observed decrease in the total number of responses on day 12 in comparison to day 1 and 4 of training. Using the combination Gal/4-AP 1:1 we recorded decrease in total number of escapes and avoidances on 12$^{th}$ day in comparison to 4$^{th}$ day ($p \leq 0,01$). In two of the ratios of combination Gal/4-AP – 1:1 ($p \leq 0,01$) and 3:1 ($p \leq 0,05$) there was statistically significant

increase in number of responses on 4th day in comparison to the same day of analyses in the control group (Figure 2).

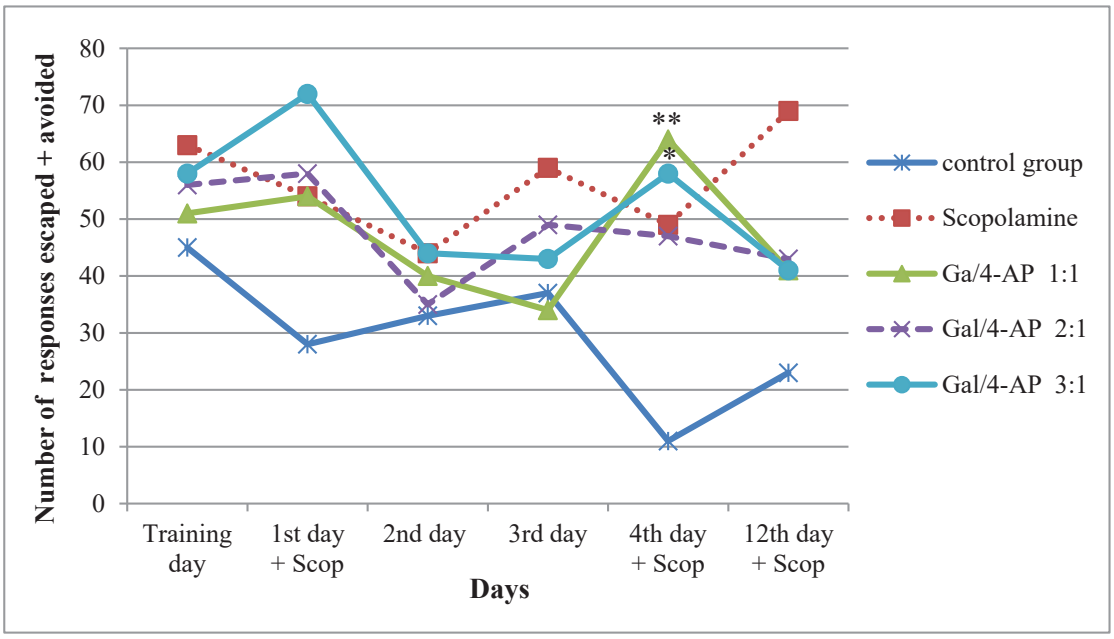


**Fig.2.** Effect of repeated administration of combination Gal/4-AP in different ratios on scopolamine-induced memory deficit in the active avoidance task.

We observed increase in number of responses escaped and avoided in groups (Figure 3), treated with combination Gal/4-AP in ratios 1:1 ($p \leq 0,05$), 2:1 ($p \leq 0,01$) and 3:1 ($p \leq 0,01$) in comparison to 4-AP. Thus, the number of responses in the galantamine-treated group decreased in comparison to ratios 1:1 ($p \leq 0,05$), and 3:1 ($p \leq 0,01$). The number of adequate responses was increased in greater extent in the group, treated with Gal/4-AP 3:1 in comparison to the other groups.

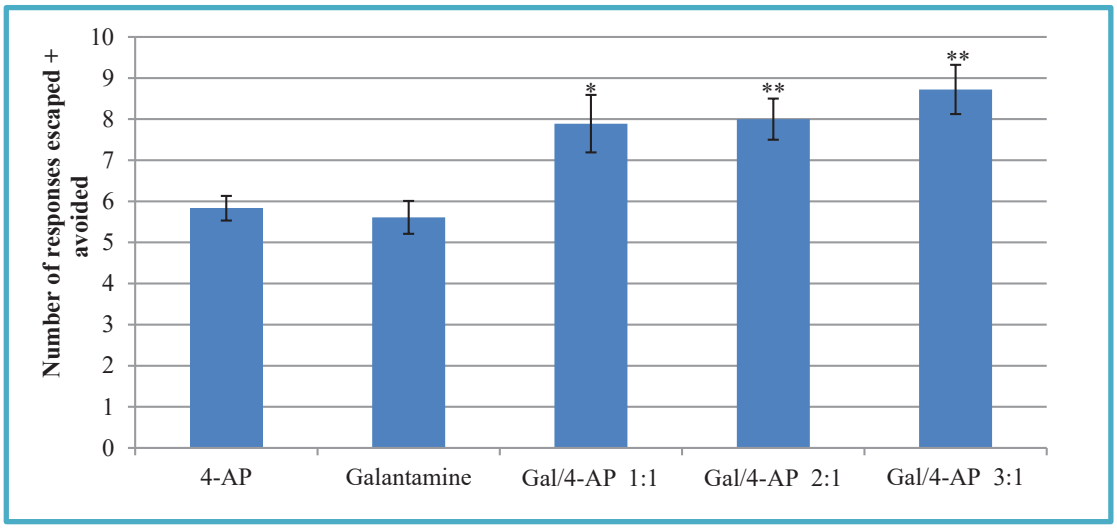


**Fig.3.** Comparison in total number of responses (escaped + avoided) after 5 measurements (days: 1, 2, 3, 4, 12).

**Disscusion**

Our results showed that combination Gal/4-AP in different ratios, as well galantamine and 4-AP didn't influence significantly the locomotor activity of mice in 1 hour of test session. We observed the same effect in 4-AP and galantamine groups, which corresponds to the data from Hernandez et al., who performed light/dark experiments to evaluate the effects of galantamine on motor function and anxiety levels that might influence memory-related behaviors. The results of these authors revealed that daily treatment with galantamine (3,6 mg/kg per day) for 12 days did not affect the general locomotor activity and anxiety-related behaviors of the aged rats [10]. The investigations of Kosaki et al. revealed that galantamine suppresses the reinstatement of methamphetamine-seeking behavior without impairing motor function or natural cravings [11]. It is known that different substances might increase or decrease locomotor activity in animals and thus could influence the results in active and passive avoidance tests. Lynch et al. reported the effects of chlorpromazine and caffeine on spontaneous locomotor activity in rats obtained from two automated test systems. Chrorpromazine decreased spontaneous locomotor activity in a dose-dependent manner, with statistically significant effects on horizontal activity at doses 8 and 16 mg/kg b.w. after single administration. By comparison, caffeine produced dose-dependent increase in spontaneous locomotor activity, with statistically significant effects on horizontal activity at doses 6, 12 and 24 mg/kg b.w. [12].

Galantamine showed activity in various behavioral tests and different models of cognitive deficit in wide dose range. Antimuscarinic drugs have been extensively used as amnesic drugs in animals to mimic the cognitive dysfunction observed in dementia and AD. The majority of the studies focused on scopolamine-reversal as initial screening method to identify therapeutic candidates for cognitive disorders. Scopolamine is a classical drug, which is used to induce a memory deficit in rodents at doses, ranging between 0,01 – 30 mg/kg. Muscarinic antagonists disrupt performance of several reference memory tasks, such as active and passive avoidance, object discrimination, radial arm maze, water maze and fear conditioning [13, 14, 15, 16].

Galantamine showed physiological cholinomimetic activity by causing hypothermia; and behavioral cholinomimetic activity by attenuating scopolamine-induced deficits in passive avoidance in mice [17]. In another study using scopolamine induced memory deficit, galantamine was tested in the T-maze (1.25, 2.5, or 5.0 mg/kg, i.p.) and in the Morris water maze (2.5 or 5.0 mg/kg, i.p.). Galantamine significantly attenuated scopolamine-induced deficits in both learning and memory models [18]. In rats studied with active and passive avoidance tasks, galantamine at 1 mg/kg but not at 0.5 mg/kg significantly improved memory retention of a learned behavior [19].

The effects of galantamine were examined in a prolonged alcohol intake model of acetylcholine deficit in male Wistar rats. After 16 weeks of alcohol intake and a 2-week pause, rats administered galantamine (2.5 mg/kg/d i.p.) showed an improved speed of learning and short-term memory in the shuttle box avoidance test as compared to the saline-injected alcoholic group. Four weeks later, significant improvement in the passive avoidance memory of alcoholic galantamine-treated rats was noted in the eight-arm radial maze (14 day test duration) as compared to the saline-injected alcoholic group. Results showed that in rats under conditions of prolonged alcohol intake galantamine improved the speed of learning, short-term memory and spatial orientation [20].

In vivo studies using non-lesioned animal models have shown that galantamine prolongs the activity of neuronally released acetylcholine and increases brain acetylcholine levels after systemic administration. Galantamine is also reported to have a strong effect in facilitating learning and memory in young and older rabbits using eyeblink classical conditioning — a form of associative learning that is severely impaired in human AD. Galantamine

has also ameliorated behavioral deficits induced by brain lesions in animal models [21].

From the Gal/4-AP combination in ratios 1:1, 2:1 and 3:1, we observed the highest effect in the 3:1 ratio resulting in a significant prolongation in latency time in a passive avoidance test and increase in the number of responses avoided in active avoidance test. In a similar combination study, it was found that using the two-way active avoidance test on rats, the best effect was achieved in Gal/4-AP 1:1 at a dose representing 1/20 of LD50 [9].

Many authors reported memory enhancing effects of well-established cholinesterase inhibitors – galantamine, rivastigmine and donepezil on scopolamine-induced memory deficit. The results of Zhang et al. demonstrated that rivastigmine (0.75 and 2.0 mg/kg) could significantly reverse the cognitive deficit in mice induced by scopolamine (0,25 mg/kg) in passive avoidance test [22]. Donepezil also reversed scopolamine-induced deficits (3 mg/kg, s.c.) in a passive avoidance task at all doses tested, across a log-scale dose-range from 0,01-1,0 mg/kg [23]. In the work of Bores and colleagues, a beneficial effect of galantamine was observed on behavior, by attenuating scopolamine-induced deficits in passive avoidance [17].

## Conclusion

In our studies the three ratios of combination galantamine/4-amiopyridine do not affect locomotor activity, which means that results from active avoidance investigations are not due to a change in motor activity in the dose regimen used. Among the presented Gal/4-AP combinations, the most beneficial effect was observed in the group, treated with 3:1 ratio.

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