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The effect of GABA transporter 1 (GAT1) inhibitor, tiagabine, on scopolamine-induced memory impairments in mice

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ABSTRACT

Background: GABAergic neurotransmission is involved in long-term potentiation, a neurophysiological basis for learning and memory. On the other hand, GABA-enhancing drugs may impair memory and learning in humans and animals. The present study aims at investigating the effect of GAT1 inhibitor tiagabine on memory and learning.
Methods: Albino Swiss (CD-1) and C57BL/6J mice were used in the passive avoidance (PA), Morris water maze (MWM) and radial arm water maze (RAWM) tasks. Scopolamine (1 mg/kg *ip*) was applied to induce cognitive deficits.
Results: In the retention trial of PA scopolamine reduced step-through latency as compared to vehicle-treated mice, and pretreatment with tiagabine did not have any influence on this effect. In MWM the results obtained for vehicle-treated mice, scopolamine-treated group and combined scopolamine + -tiagabine-treated mice revealed variable learning abilities in these groups. Tiagabine did not impair learning in the acquisition trial. In RAWM on day 1 scopolamine-treated group made nearly two-fold more errors than vehicle-treated mice and mice that received combined scopolamine and tiagabine. Learning abilities in the latter group were similar to those of vehicle-treated mice in the corresponding trial block on day 1, except for the last trial block, during which tiagabine + scopolamine-injected mice made more errors than control mice and the scopolamine-treated group. In all groups a complete reversal of memory deficits was observed in the last trial block of day 2.
Conclusions: The lack of negative influence of tiagabine on cognitive functions in animals with scopolamine-induced memory impairments may be relevant for patients treated with this drug.

Introduction

The termination of GABA action is mediated by its neuronal or astrocytic re-uptake. The majority of released GABA is transported into presynaptic nerve endings, whereas a smaller fraction is moved into astrocytes associated with these synapses. GABA taken up into presynaptic nerve endings is re-utilized as a neurotransmitter, but it can also be metabolized, both in neurons and astrocytes [1].

Until now, four plasma membrane GABA transporters (GAT) implicated in GABA re-uptake have been identified, cloned and thoroughly investigated as a potential drug target for the treatment of numerous neurological and psychiatric disorders [2]. In mice these transport proteins are named GAT1-4, whereas in rats and humans they are named GAT-1, BGT-1, GAT-2 and GAT-3, respectively [3].

Among numerous GAT inhibitors that have been synthesized and studied [3–14], there is only one drug that has been introduced into clinic, so far. Tiagabine (Fig. 1), a selective GAT1 inhibitor with IC_{50} of 0.11 μM [10], is used as an add-on therapy of partial seizures in men. Recent animal [15,16] and human [17–21] studies have demonstrated that it can be also effective in the treatment of chronic pain, anxiety or depression. Adverse effects of tiagabine

Abbreviations: CNS, central nervous system; GABA, γ-aminobutyric acid; GAT, GABA transporter; MWM, Morris water maze; PA, passive avoidance; RAWM, radial arm water maze.

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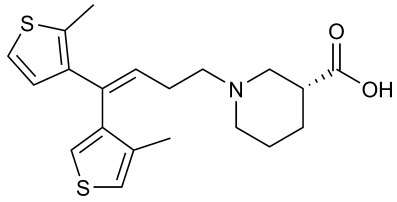

Fig. 1. Chemical structure of tiagabine.

comprise numerous CNS-derived effects, such as sedation, asthenia, dizziness and tremor [22,23].

A number of neurotransmitters differentially involved in the formation and retrieval of memory have been studied [24], and the neurotransmitters, such as glutamate, GABA, dopamine and acetylcholine have been reported to have more powerful impact (81–93%) on cognitive processes than serotonin and norepinephrine (48–55%). In neurodegenerative disorders which affect memory processing, pathological changes have been reported to be related to glutamatergic, cholinergic, noradrenergic and serotonergic neurotransmitter systems [24]. Noteworthy, not only glutamate, but also GABA and GABA-A receptors are involved in long-term potentiation, a phenomenon which is considered a neurophysiological basis for learning and memory processes [24–28]. Moreover, Shi et al. [27] showed that a moderate reduction of GAT1 activity caused cognitive enhancement in GAT1 heterozygous mice. On the other hand, some GABAergic drugs with anticonvulsant properties have been found to seriously impair learning and memory, both in humans [23,29] and experimental animals [30]. In view of these conflicting data, it seems interesting to investigate the effect of tiagabine on cognition. Current literature devoted to the influence of this drug on learning and memory is very limited. Hence, in the present study using three behavioral assays, *i.e.*, the passive avoidance test (PA) which is a fear-motivated task, and two tasks assessing spatial memory in rodents: Morris water maze (MWM) and radial arm water maze (RAWM), we have investigated the potential impact of this GAT1 inhibitor on learning and memory. We have used scopolamine, a nonselective cholinergic M receptor antagonist, a 'gold standard' drug for the induction of cognitive deficits in animals. This drug induces age- and dementia-related cognitive deficits in animals [31]. These cognitive impairments can be recognized by means of several 'land tasks' (*e.g.*, PA task) and 'water maze tasks' (*e.g.*, MWM or RAWM) [32].

Materials and methods

Animals

Eight-week old male Albino Swiss (CD-1) mice weighing between 18 and 22 g were used in the PA test, and C57BL/6J mice were used in the MWM and two-day RAWM tests. For each of these tasks separate groups of mice were used to avoid the possibility that one test may affect the results of another. The animals were housed in groups of 10 mice per cage at room temperature of 22 ± 2 °C, under light/dark (12:12) cycle. The animals had free access to food and water before experiments. The ambient temperature of the room and humidity were kept consistent throughout all the tests. For behavioral experiments the animals were selected in a random way. Each group consisted of 8–10 animals/dose, and each mouse was used only once. The experiments were performed between 8 a.m. and 2 p.m. Immediately after *in vivo* assays the animals were euthanized by cervical dislocation. The maintenance and treatment of laboratory animals were carried out in accordance with guidelines issued by the Local Ethics Committee of the Jagiellonian University in Cracow (ZI/862/2013).

Chemicals used in behavioral assays

Tiagabine (doses: 10 and 30 mg/kg in PA, and 10 mg/kg in MWM and RAWM) was purchased from Tocris Bioscience (Germany). For *in vivo* experiments it was suspended in 1% Tween 80 (Polskie Odczynniki Chemiczne, Gliwice, Poland) and administered intraperitoneally (*ip*) 60 min before the test (for a detailed protocol of drug administration see "Behavioral testing paradigm" section). Control mice were given appropriate amount of vehicle (1% Tween 80). (−)-Scopolamine hydrochloride was purchased from Sigma–Aldrich (Poland). To induce memory impairments it was dissolved in distilled water and administered *ip* at a dose of 1 mg/kg 30 min before the tests.

Behavioral testing paradigm

Passive avoidance task

The effect of tiagabine on acquisition and retention of PA task was conducted according to a previously described method [33]. For this purpose the passive avoidance apparatus (Panlab Harvard Apparatus, Barcelona, Spain) was used. It consists of a large white-painted illuminated compartment (26 cm × 26 cm × 34 cm) and a small black-painted dark compartment (13 cm × 7.5 cm × 7.5 cm) separated from each other by a guillotine gate.

To assess the effect of tiagabine on scopolamine-induced memory impairments the animals underwent two separate trials: an acquisition trial (conditioning phase) and a retention trial (testing phase). The latter was conducted 24 h after the acquisition trial. One hour before the acquisition trial, the mice were pretreated with tiagabine or vehicle. Control animals received 1% Tween 80 solution. Thirty minutes later, the animals were treated with scopolamine hydrochloride. For the acquisition trial, each mouse was initially placed for 30 s in the light compartment (exploration period; guillotine gate is closed). At the end of the exploration period the guillotine door (5 cm × 5 cm) between the light and the dark compartments was opened and the time that elapsed before entering the black chamber was recorded. As soon as the mouse entered the dark compartment, the door automatically closed and an electrical shock (current intensity: 0.2 mA, duration: 2 s) was delivered through the grid floor.

For the retention trial, the mice were placed in the illuminated white compartment again, and the latency time between door opening and entry into the dark compartment was recorded for each mouse. If the mouse did not enter the dark compartment within 180 s (cut off latency), it was concluded that it remembered the foot shock from the acquisition trial. Better memory performance was indicated by longer latency to enter in the black chamber in the test (retention) phase than in the conditioning (acquisition) phase.

Morris water maze test

The MWM (Panlab Harvard Apparatus, Spain) is a circular, plastic and gray-painted pool (120 cm in diameter and 60 cm in height), filled with water (up to about 48 cm below the edge to prevent an animal jumps out) maintained at 23 ± 1 °C. The pool was divided into four equal quadrants (compass locations: NE, NW, SE, SW) by a computerized video tracking system (SMART, ver. 3.0; Panlab, Spain). An escape platform (11 cm in diameter and 47 cm in height) at a fixed location (the center of the NW quadrant, *i.e.*, the target quadrant) was made of transparent Plexiglas, invisible to the swimming animal and was immersed 1 cm under the surface of water. The maze was lighted with the intensity of 45 lx.

During the spatial acquisition trial (6 consecutive days) mice were assigned to training sessions (four training sessions a day; sessions were held at 4 h intervals) in which the mice were trained to escape from water by reaching a hidden platform whose

location could be identified using distal extra-maze cues (A4-size sheets of black laminated paper with color geometric symbols) attached to the room walls and constituted navigation points [34]. Visual cues had different colors and dimensions and were kept constant during the whole experiment [32,34]. The whole experiment was conducted by an experimenter who remained always stationary in a constant location being an additional, distal cue for swimming animals. For each trial the animal was placed in the water starting from a different, randomly chosen quadrant that did not contain the platform, whereas the platform was always positioned in the same place. If an animal did not find the hidden platform within 60 s, it was gently placed on the platform for 15 s. The time taken to reach the hidden platform (escape latency time), distance traveled to reach the hidden platform, distance (%) in the target (NW) zone, and mean speed were recorded. The strategy used during trials was also recorded and analyzed.

On the seventh day (24 h after the last training) the platform was removed from the pool and a probe trial was performed (without drug treatment). Each animal was released from a different start point and was allowed to swim for 60 s. If a mouse did not find the platform's place within 60 s, it was given a latency score of 60 s [32,35]. Latency time to the first crossing of the former platform location (target zone), the number of crossings of the target zone, time spent in the target NW quadrant, total distance, the distance spent in NW quadrant, entries in NW quadrant and mean speed were measured. The traveled trajectories were tracked and analyzed, as well [34].

Two-day radial-arm water maze

C57BL/6J mice were subjected to a two-day RAWM paradigm [36,37]. The main part of the apparatus (Panlab Harvard Apparatus, Spain) consists of a 6-arm maze located in the pool filled with water to approximately 10 cm from the top to be high enough to cover the hidden platform by about 0.5 cm, but also low enough that the visible platform is not covered. The pool was regularly cleaned of any debris left by the animals. The temperature of water was kept constant during testing (23 ± 1 °C). The tester remained in the same position throughout testing, being an additional visual cue for the animals.

On day 1, 15 trials in five blocks of 3 were conducted. Each trial lasted up to 60 s. The start arm was varied for each trial, with the goal arm remaining constant for a given individual for both days [36,38]. For the first 12 trials, the platform was alternately visible, then hidden, and hidden for the last 3 trials. On day 2, the experiments (without drug treatment) were run in the same manner as on day 1 but the platform was hidden for all trials. Since errors have been found to be the most sensitive measure in this assay [36], the number of errors (*i.e.*, incorrect arm entries made) was counted in a 60 s frame. Incorrect arm entries occurred when the mouse selected an arm that was not the goal arm. Entries into the goal arm were not counted as errors even, if the platform was not located. An entry was considered to occur when all four legs of the animals have entered the alley completely. A failure to select an arm after 15 s was counted as an error, and the mice that failed to make an arm choice in 15 s were assigned one error. If the platform was not located during 60 s, the mice were gently guided through the water by placing a hand behind the animal to direct its swimming direction toward the platform. If the mouse located the platform within 60 s or was guided toward the platform, it was allowed to stay on the platform for 15 s. After that, the mouse was gently removed from the platform and thoroughly dried using cellulose paper before placing it back into its home cage under a heat source (a heat lamp).

For the statistical analysis of the results obtained, in order to minimize the impact of individual trial variability, each mouse's errors to find the platform for 3 consecutive trials were averaged giving 5 data points (5 trial blocks): T1–T3; T4–T6; T7–T9; T10–T12; T13–T15 separately for each day of testing.

Results

Passive avoidance task

In this fear-motivated task the effect of tiagabine on scopolamine-induced cognitive dysfunction was assessed. A significant overall effect of treatment was observed ($F[7,70] = 13.15$; $p < 0.0001$). In the acquisition trial, we did not observe significant inter-group differences in the step-through latency between vehicle-treated mice and scopolamine-treated group, as well as between scopolamine-treated mice and tiagabine-treated memory-impaired mice ($p > 0.05$). Statistically significant differences were observed in the acquisition trial between vehicle-treated mice and mice that received combined scopolamine and tiagabine ($p < 0.001$ for tiagabine 10 mg/kg, and $p < 0.01$ for tiagabine 30 mg/kg). In the retention trial the step-through latency of scopolamine-treated mice was significantly shorter compared to vehicle-treated control animals ($p < 0.001$). However, in the retention trial the reduction of step-through latency caused by scopolamine was not affected by tiagabine (Fig. 2).

Morris water maze

Effect on acquisition

During the training days the latency to reach the hidden platform was measured and analyzed with repeated-measures ANOVA. Latencies to reach the target platform progressively decreased during the six training days, which produced characteristic learning curves for all groups tested. The results obtained for vehicle-treated mice, scopolamine-treated mice and combined scopolamine + tiagabine-treated mice revealed variable learning abilities in these experimental groups (Fig. 3). A significant drug effect was shown ($F[2,120] = 9.76$; $p < 0.001$). Time also affected the results in a statistically significant manner ($F[5,120] = 58.00$; $p < 0.0001$).

During the training days the distance traveled to reach the hidden platform was also measured and analyzed (Table 1). The results showed significant differences between groups (drug effect: $F[2,96] = 26.14$; $p < 0.0001$). Time also influenced learning abilities ($F[5,48] = 33.90$; $p < 0.0001$).

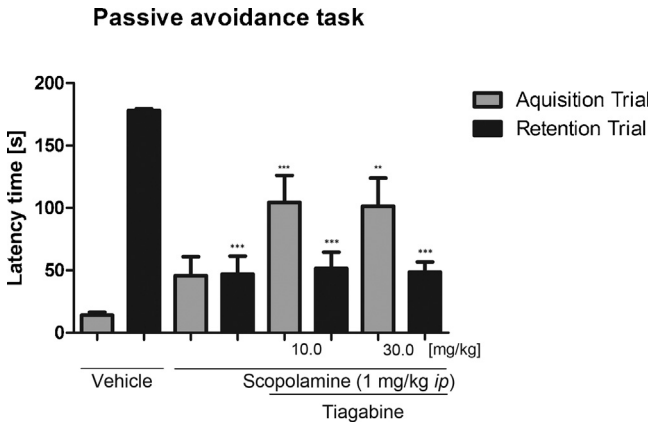

Fig. 2. Effect of tiagabine on scopolamine-induced memory impairments measured using passive avoidance task. Results are shown as the mean latency time [s] ± SEM to enter the dark compartment in the acquisition trial (gray bars), and in the retention trial (black bars). Statistical analysis: one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Significance *vs.* vehicle-treated mice: $^{**}p < 0.01$, $^{***}p < 0.001$ (acquisition trial); $^{***}p < 0.001$ (retention trial).

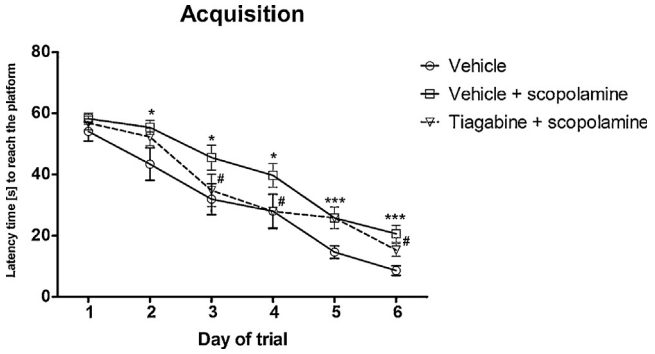

Fig. 3. Learning curves showing the acquisition phase in vehicle-treated mice, scopolamine-treated mice, and mice treated with combined scopolamine + tiagabine. Results are shown as mean escape latency time [s] ± SEM. Statistical analysis: repeated-measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance: $^{*}p < 0.05$, $^{***}p < 0.001$ (*vs.* vehicle-treated mice); $^{\#}p < 0.05$ (*vs.* scopolamine-treated mice).

The analysis of distance (%) traveled in the NW (target) zone during days 1–6 revealed a significant drug effect ($F[2,24] = 7.83$; $p < 0.01$) and time effect ($F[5,120] = 12.70$; $p < 0.0001$) on this parameter. On day 1 the distance (%) traveled in the NW quadrant was similar in all tested groups (22–24%). Starting on day 3, the distance in NW zone measured in vehicle-treated mice and mice treated with combined scopolamine and tiagabine was similar (31% and 35%, respectively on day 3, and 41–44% in both groups on days 4–6). For scopolamine-treated group the distance (%) traveled in NW quadrant was 27% on day 3, and 32–33% on days 4–6.

The mean speed during training days was also analyzed in all groups. Significant drug ($F[2,24] = 3.73$; $p < 0.05$) and time ($F[5,120] = 4.66$; $p < 0.001$) effects were shown (Table 1).

The strategy used during training trials was recorded (Fig. 4) and analyzed by categorizing each individual trial according to the predominant swim pattern. Several categories were defined to capture the gradually improving spatial precision and efficiency during the learning process [32]. For the classification of swimming behavior the following criteria were applied [32]: mice naïve to the MWM initially tended to swim along the wall of the pool ('wall hugging'; Fig. 4A–C). With the training progress, mice started to search the whole pool surface, first randomly (Fig. 4D–F, H, N, O) and then selectively scanning the inner area of the pool that contained the escape platform (Fig. 4G, K). The progress of the learning process and the development of spatial memory to localize the hidden platform was reflected by a focal search for the target quadrant (Fig. 4K, Q) or swimming directly to the platform (Fig. 4I, J, L, M, P, R).

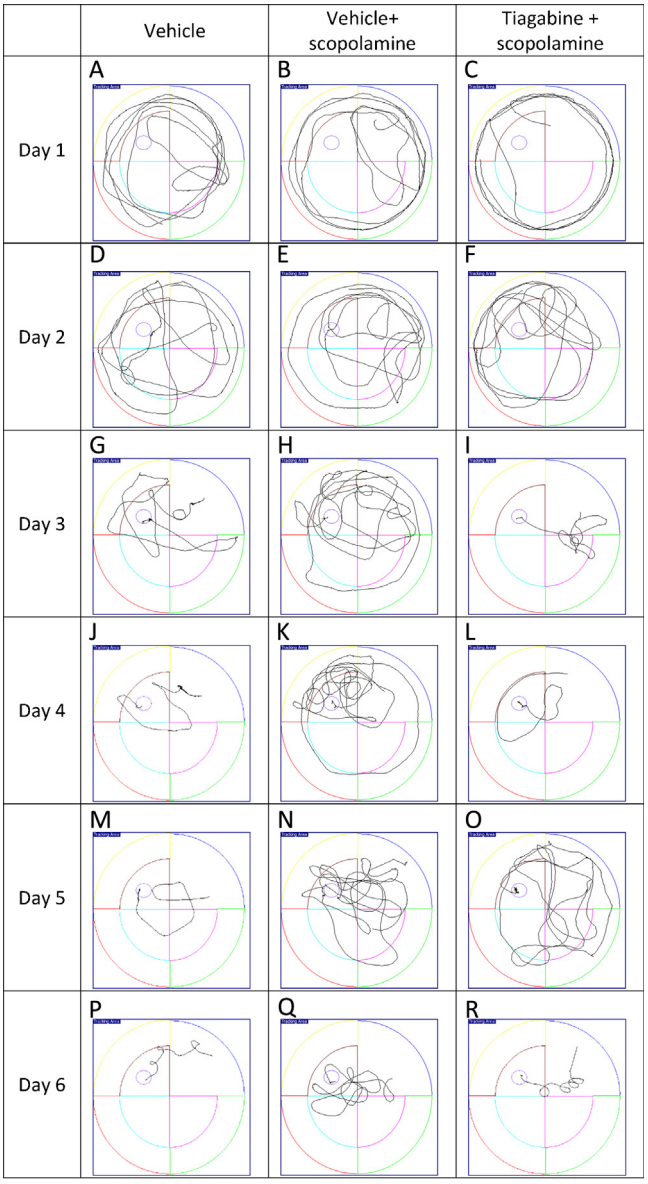

Fig. 4. Examples of swim patterns (escape strategies) during the acquisition phase of the MWM test in vehicle-treated mice, scopolamine-treated mice, and mice treated with combined scopolamine and tiagabine. Trajectories are shown for the last trial on each training day.

Table 1
Effect of tiagabine on memory acquisition in MWM measured using selected parameters.

Group	Parameter measured	Training day					
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Vehicle	Distance [m] to platform (±SEM)	9.2 ± 0.6	7.4 ± 0.8	3.7 ± 0.9	3.6 ± 0.7	1.9 ± 0.5	1.4 ± 0.1
	Mean speed [m/s] (±SEM)	0.17 ± 0.01	0.15 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.18 ± 0.02
Vehicle + scopolamine	Distance [m] to platform (±SEM)	10.6 ± 0.6	11.0 ± 0.6**	8.0 ± 0.8***	6.6 ± 0.6*	4.3 ± 0.6*	3.9 ± 0.5*
	Mean speed [m/s] (±SEM)	0.17 ± 0.01	0.19 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.24 ± 0.09
Tiagabine + scopolamine	Distance [m] to platform (±SEM)	9.5 ± 0.7	10.4 ± 0.8*	5.9 ± 1.2	4.3 ± 1.1	4.1 ± 0.9	2.4 ± 0.6
	Mean speed [m/s] (±SEM)	0.17 ± 0.01	0.18 ± 0.1	0.14 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.01

Statistical analysis: repeated-measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance *vs.* vehicle-treated mice.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

Table 2
Effect of tiagabine on memory retention (the probe trial on day 7) measured in MWM.

Parameter analyzed	Vehicle	Vehicle + scopolamine	Tiagabine + scopolamine
Latency time [s] ± SEM to the first crossing of the former platform location (target zone)	42.03 ± 4.4	37.14 ± 2.8	37.21 ± 4.4
Time [s] ± SEM spent in the target (NW) quadrant	26.51 ± 2.6	22.29 ± 1.7	22.33 ± 2.6
Mean speed [m/s] (±SEM)	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Number of NW zone crossings (±SEM)	4.0 ± 0.6	3.11 ± 0.6	2.77 ± 0.7
Distance (%) in goal-quadrant (±SEM)	42.69 ± 3.8	36.25 ± 2.9	38.17 ± 4.5
Entries in zone NW (±SEM)	13.11 ± 1.3	11.56 ± 0.8	9.9 ± 0.9
Total distance [m] (±SEM)	10.9 ± 0.3	11.1 ± 0.4	10.5 ± 0.6

Results shown for $n = 9$ animals per group. Statistical analysis: one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test. Significance *vs.* scopolamine-treated control – not significant.

Effect on memory retention (probe trial on day 7; short-term retention phase)

During the probe trial (off drug) the latency time to the first crossing of the former platform location (target zone), the number of crossings of the target zone, time spent in target NW quadrant, total distance, the distance in NW quadrant, entries in NW quadrant and the mean speed were measured in all groups and analyzed (Table 2). The latency time to the first crossing of the former platform location was similar in all tested groups ($F[2,25] = 0.5099$; $p > 0.05$). The time spent in the target quadrant on day 7 was compared and the differences were not statistically significant ($F[2,24] = 1.075$; $p > 0.05$). Mean speed in each group was measured and the results obtained were similar in all tested groups ($F[2,24] = 0.1304$; $p > 0.05$).

During the probe trial on day 7, the number of target zone crossings was also measured ($F[2,24] = 1.021$; $p > 0.05$). The distance traveled in the target NW quadrant and in the average adjacent quadrants of the MWM was measured and analyzed. The differences among groups were not statistically significant ($F[2,24] = 0.7620$; $p > 0.05$). The number of entries in NW target zone and the total distance traveled were similar in all tested groups ($F[2,24] = 2.377$; $p > 0.05$; $F[2,24] = 0.2692$; $p > 0.05$, respectively). However, the analysis of searching strategies in all groups tested revealed a greater tendency to explore the NW quadrant in vehicle-treated mice (Fig. 5A) compared to two other experimental groups (Fig. 5B, C).

Two-day radial-arm water maze

Effect on acquisition

The two-day RAWM was used for the assessment of spatial navigation learning and memory deficits in vehicle-treated mice, scopolamine-treated mice and mice that were injected combined scopolamine and tiagabine. In the RAWM task the vehicle-treated mice began the test naïve and made an average of 4 errors per the first trial block (Fig. 6). These mice were making less than one error by the end of the first day. Comparing learning abilities in each trial block in the experimental groups (Fig. 6) we showed that on day 1 scopolamine-treated group made nearly two-fold more errors than vehicle-treated mice and mice that received combined scopolamine and tiagabine (drug effect: $F[2,24] = 5.21$; $p < 0.05$; trial block effect: $F[4,96] = 13.64$; $p < 0.0001$). Learning abilities of the latter group were similar to those of vehicle-treated mice in the corresponding trial block on day 1, except for the last trial block, during which combined tiagabine + scopolamine-injected mice made more errors (less than 3) than control mice (less than 1 error) and scopolamine-treated group (less than 2 errors).

Effect on memory retention

On day 2 of the RAWM, in trials T1–T12 the vehicle-treated mice and mice treated with combined tiagabine + scopolamine displayed similar cognitive capabilities that were superior to those of scopolamine-treated group (drug effect: $F[2,24] = 2.80$;

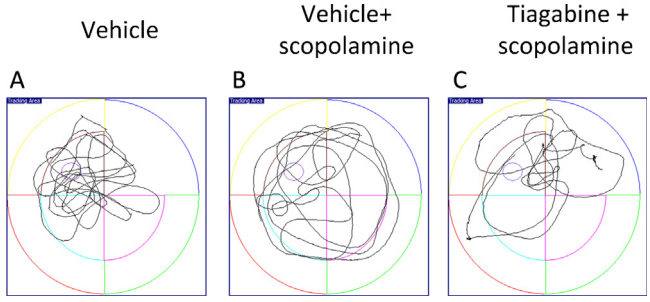

Fig. 5. Example of searching strategies during the probe trial (day 7) of the MWM test in vehicle-treated mice, scopolamine-treated mice, and mice treated with combined scopolamine and tiagabine.

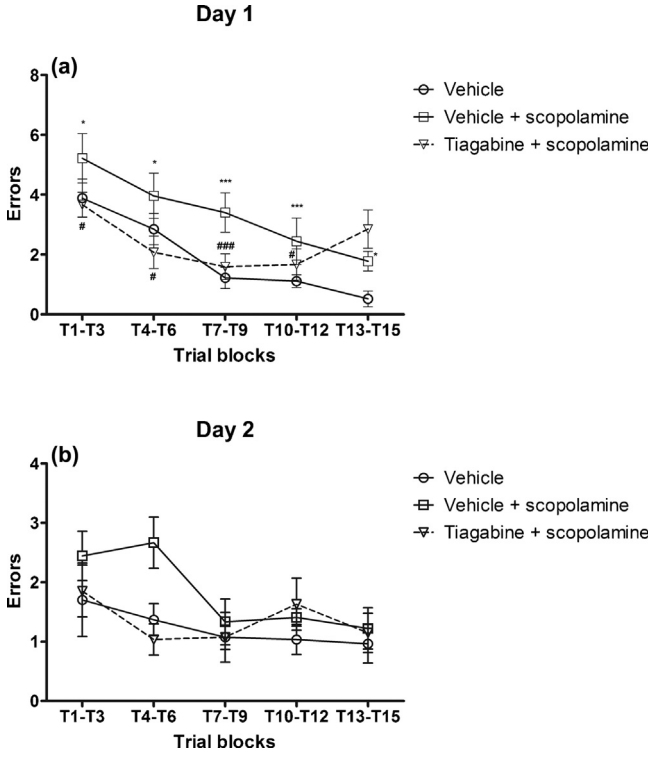

Fig. 6. Spatial learning deficits expressed as the mean number of errors (±SEM) made in each trial block on day 1 of the two-day RAWM, and day 2 of the two-day RAWM. Statistical analysis: repeated-measures ANOVA, followed by Bonferroni *post hoc* comparison. Significance: $^{*}p < 0.05$, $^{***}p < 0.001$ (*vs.* vehicle-treated mice); $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ (*vs.* scopolamine-treated mice).

$p > 0.05$; trial block effect: $F[4,96] = 3.32$; $p < 0.05$; Fig. 6). In all groups a reversal of memory deficits was observed in the last trial block of day 2, and each group completed RAWM task with a mean performance near 1 error per the last trial block.

Discussion

The impairments of learning and memory (cognitive dysfunction) might be due to several reasons, such as advanced age, neurodegenerative disorders, chronic stress and others [39]. In addition, a relatively high percent of patients present memory deficits induced by centrally-acting drugs. In the recent years accumulating data regarding antiepileptic drug-induced memory impairments and cognitive dysfunctions have been gathered [40]. Importantly, cognitive dysfunction is frequently observed in patients with epilepsy, so it represents an important challenge in the management of patients with this disorder. In this respect, the contribution of antiepileptic drugs to cognitive deficits in epileptic patients is of relevance, as it can potentiate memory impairments that result from the disease itself. The fact that a considerable number of patients require antiepileptic drug therapy for many years, or perhaps even a lifetime, emphasizes the need to assess the effects of this pharmacological class on cognition [41].

Numerous neurotransmitters, including glutamate, dopamine, serotonin and GABA are implicated in learning and memory [28], but the role of their specific transport proteins and uptake inhibitors is much less known and rather conflicting [28]. For instance, it was shown that tiagabine impaired memory in MWM [30], while another potent GAT1 inhibitor [42], namely NNC-711, was able to prevent scopolamine-induced amnesia in the PA task [43]. Moreover, GAT1 knock-out mice exhibited impaired hippocampus-dependent memory [44], while the up-regulation of GAT1 impaired associative learning and novel object recognition retention which were reverted by the chronic administration of GAT1 inhibitor [45]. On the other hand, it was also shown that memory formation was associated to prefrontal cortex GAT1 up-regulation and down-regulated hippocampal GAT1. The facilitation of memory involved up-regulation of GAT1 and DAT [28], whereas O'Connell et al. [43] demonstrated that GABA transport inhibitors may have anti-amnestic properties and may be cognitive enhancers.

PA test, a fear-motivated avoidance task, is one of the most abundantly used behavioral methods to measure cognitive abilities of drug candidates in rodents [46], and scopolamine is a 'gold standard' drug for inducing cognitive deficits in animals [31]. In PA task drugs that affect GABAergic neurotransmission have weaker impact on animal performance than these affecting dopaminergic or glutamatergic systems [24] but increased retention latency in GAT1$^{+/-}$ mice was shown in PA [27], which indicates for the enhancement of amygdala- and hippocampus-dependent emotional memory encoding. These authors showed that GAT1$^{+/-}$ mice displayed improved cognitive ability and decreased anxiety-related behavior compared to wild-type mice [27].

In the acquisition phase of the PA test statistically insignificant differences between step-through latencies were observed in vehicle-treated mice and scopolamine-treated control animals. Statistically significant differences were observed in this phase between mice injected with vehicle (alone or in combination with scopolamine) and mice that received combined tiagabine and scopolamine. The latter group had prolonged latency to enter the dark compartment of the PA apparatus. A possible explanation of this finding is that at doses tested in PA tiagabine demonstrates sedative properties, reduces spontaneous locomotor activity and may impair motor coordination in the rotarod test [15]. Although drugs affecting locomotor activity and drugs that reduce fear are expected to cause impairments of the PA test [24], the impact of the latter should be excluded from our study, as previously we showed [15] that tiagabine at the dose of 8 mg/kg demonstrated anxiolytic-like properties in the four-plate test and increased the number of 'punished crossings'. In view of this, it seems that prolonged step-through latency in the acquisition trial results from sedation caused by tiagabine. In our previous research (unpublished results) we also tested the impact of scopolamine on animals' locomotor activity and we showed that at the dose of 1 mg/kg (*ip*) scopolamine-induced decrease in locomotor activity was not statistically significant compared to control group, so its contribution to the effects observed in PA task is rather implausible.

The results from the PA task revealed significant prolongation of retention trial latency in comparison to the acquisition trial in vehicle-treated mice which indicates for unimpaired learning and memory in these mice. Significant reduction of step-through latencies in scopolamine-treated group (46.9 ± 14.5 s) in the retention trial compared to vehicle-treated mice (178.1 ± 1.3) indicates for scopolamine-induced memory deficits. The comparison of retention trial step-through latency of vehicle-treated mice and retention trial latency of mice treated with combined scopolamine and tiagabine (51.6 ± 13.0 for 10 mg/kg, and 48.6 ± 8.1 s for 30 mg/kg) shows learning deficits in the latter group. However, no differences between retention trial latencies of scopolamine-treated mice and retention trial latencies of combined scopolamine and tiagabine-treated animals were observed, which proves that in this test scopolamine reduced step-through latency as compared to vehicle-treated mice, and the pretreatment with tiagabine did not have any influence on this effect.

The GABAergic system plays a key role in the adequate performance in the MWM which is frequently used for the evaluation of spatial learning and memory in rodents [47]. The activation of GABAergic neurotransmission is thought to interfere with spatial learning abilities in this test. It was demonstrated that the suppression of GABA function may enhance spatial learning [48], while the enhancement of GABAergic neurotransmission has adverse impact on MWM behavior [24]. Both MWM and PA involve working memory and reference memory [24]. MWM permits to study reference memory, spatial learning and working memory [47,49]. To assess the impact of tiagabine on spatial memory we used C57BL/6 mice which show better performance in MWM than CD-1 mice [32,34,47,48] due to visual impairment of the latter strain [34]. Earlier studies demonstrated that subchronic administration of vigabatrin, a drug that enhances GABAergic neurotransmission, did not influence learning performance in the MWM [24,48]. On the other hand, diazepam and triazolam affected acquisition but not recall of spatial information in the MWM [48], while GABA-B receptor antagonist, CGP-36742 attenuated baclofen- and scopolamine-induced MWM deficits [48]. It was also shown that GABA-B receptor antagonist CGP-46381 affected acquisition in MWM, without any influence on working memory performance in the radial-arm maze [48]. These facts indicate a strong involvement of the GABAergic system in learning and memory in the MWM.

In this study tiagabine compared to vehicle (mice without scopolamine treatment) prolonged the latency to find the hidden platform during the acquisition phase, but this difference was not statistically significant. In tiagabine-treated mice at the end of the acquisition phase the total distance swum was not affected, either. In contrast to this, in scopolamine-treated group an increase in the total distance traveled was observed, which indicates for impaired learning in these mice. Statistically significant differences of distance traveled to find the platform were shown for vehicle-treated mice and scopolamine-treated mice. This effect was observed starting from the second day of acquisition phase.

Analyzing the percent of distance traveled in the target NW quadrant it was shown that both vehicle-treated mice and mice treated with combined tiagabine and scopolamine had similar values, while for scopolamine-treated group these percentage values were lower. This indicates impaired learning in scopolamine-treated mice and demonstrates unimpaired learning and memory in two other tested groups. The analysis of the mean speed values in vehicle-treated mice and mice that received combined scopolamine and tiagabine revealed a decrease in the mean speed starting from day 3 of the acquisition period. Such effect was not observed in scopolamine-treated group, which might indicate that scopolamine did not affect motivational behavior or locomotor activity in MWM.

Stress related to water immersion is one of the crucial factors for behavior in MWM, so it should be noted that the use of GABA-enhancing drugs giving anxiolytic and depressive effects in the CNS could significantly affect learning and memory tests in animals [47]. In view of this, the observed in MWM prolongation of latency time to reach the platform in tiagabine-treated mice compared to vehicle-treated group can be explained taking into account the previously shown [15] anxiolytic-like and sedative properties of tiagabine at similar dose range.

In contrast to results obtained by Schmitt and Hiemke [30], our study did not reveal negative effect of tiagabine on learning abilities in MWM. This discrepancy can be explained by the fact that these authors used a two-fold higher dose than we did. In our research the selection of a dose of 10 mg/kg was dictated by the activity observed in other tests in mice [15].

Taken together, the results obtained in the MWM task indicate that vehicle-treated mice had unimpaired spatial learning abilities, while scopolamine induced learning deficits in mice. No differences between vehicle-treated mice and mice treated with combined scopolamine and tiagabine were observed, which proved that in this test tiagabine did not exert a negative effect on spatial learning in mice. These results also indicate that during the acquisition trial pretreatment with tiagabine either prevented or attenuated scopolamine-induced memory impairments. The putative mechanism of this beneficial effect is not clear, yet it indicates a potential interaction between the cholinergic and GABAergic neuronal circuits. The hypothesis that the enhancement of GABAergic neurotransmission (due to tiagabine-evoked GAT1 inhibition) can, at least partially, abolish cholinergic blockade induced by scopolamine, requires further studies, though this mechanism seems to underlie the effects that were observed *in vivo*. Our results remain in agreement with those obtained by other authors [43] who demonstrated that GAT1 inhibitors prevented scopolamine-evoked amnesia.

Analyzing the searching strategy it was revealed that the vehicle-treated mice had the best learning abilities during subsequent days of training. Tiagabine-treated mice learned slower than vehicle-treated mice but more quickly than the scopolamine-treated group.

During the probe trial of the MWM spatial accuracy of animals was determined, being represented by the time spent in the quadrant where the platform was during the acquisition phase, or by the number of times the animal crossed the former platform area. Well-trained animals show high preference for the target quadrant, and spend about 50% or more of their free swimming time scanning this quadrant [48]. The results obtained for the retention phase revealed that scopolamine had no influence on the parameters measured, which is consistent with previous studies showing that in MWM systemic administration of scopolamine had stronger effect on disrupting acquisition than impairing retention [31]. Latency to first entrance to target NW zone, time spent in it, number of target zone crossings, distance in NW zone, total distance swum, mean speed and number of entries in NW quadrant on day 7 were at similar range in all experimental groups. Searching strategies were also similar in these groups, however it seems that vehicle-treated mice more preferentially chose NW quadrant than two other groups, in particular the scopolamine-treated group.

RAWM is a test to measure spatial learning and memory, being a combination of dry radial arm mazes and MWM. The advantages of RAWM comprise the ability to combine a complex spatial environment with an easy way of measuring animal performances by counting errors without a need to use a video tracking system or computer, to give animals a strong motivation (to escape from water) without requiring foot deprivation or foot shock, and possibly test both working memory and reference memory [34]. In a two-day RAWM test the effect of tiagabine on reference memory was tested [34]. On day 1 we showed impaired learning in scopolamine-treated mice for which errors were double those made by both vehicle-treated mice and mice that received combined tiagabine and scopolamine. Analyzing good spatial performance of mice that received combined tiagabine and scopolamine (the number of errors was similar to values of vehicle-treated mice), it was shown that, similarly to results obtained in MWM, tiagabine not only had no negative effect on working memory in RAWM, but it also prevented or attenuated scopolamine-induced memory deficits. On day 2 all mice demonstrated a reversal of memory impairments as observed in the last trial block of day 2, ending this day with a mean performance near 1 error per trial block. It can be therefore concluded that in RAWM, similarly to MWM, scopolamine did not disrupt memory retention, but it influenced acquisition.

In conclusion, so far there are limited data about the influence of tiagabine on learning and memory in rodents. To the best of our knowledge, this study is the first one which assessed the effect of tiagabine in memory-impaired mice using three different tasks. We demonstrated that in the retention phase of PA task the reduction of step-through latency caused by scopolamine was not influenced by tiagabine. The results obtained in MWM and RAWM indicated that the pretreatment with tiagabine in the acquisition trial partially prevented or attenuated, scopolamine-induced memory impairments. This potentially beneficial effect of the investigated GAT1 inhibitor on cognitive functions shown in behavioral tests is relevant not only in terms of epileptic patients treated with tiagabine, but also those who use this drug for other therapeutic indications.

Conflict of interest

None declared.

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