

## Research report

## PWZ-029, an inverse agonist selective for $\alpha_5$ GABA<sub>A</sub> receptors, improves object recognition, but not water-maze memory in normal and scopolamine-treated rats

Marija Milić<sup>a</sup>, Tamara Timić<sup>a</sup>, Srđan Joksimović<sup>a</sup>, Poonam Biawat<sup>b</sup>, Sundari Rallapalli<sup>b</sup>, Jovana Divljaković<sup>a</sup>, Tamara Radulović<sup>a</sup>, James M. Cook<sup>b</sup>, Miroslav M. Savić<sup>a,\*</sup>

<sup>a</sup> Department of Pharmacology, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11221 Belgrade, Serbia

<sup>b</sup> Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, WI 53201, USA

## HIGHLIGHTS

- ▶ PWZ-029 is an  $\alpha_5$  GABA<sub>A</sub> selective inverse agonist.
- ▶ We tested PWZ-029, on its own and with scopolamine, in two memory paradigms.
- ▶ It improved object recognition in the novel object recognition test.
- ▶ PWZ-029 was ineffective in the Morris water maze test.
- ▶ Testing of putative procognitive compounds needs use of a variety of models.

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## ABSTRACT

Inverse agonism at the benzodiazepine site of  $\alpha_5$  subunit-containing GABA<sub>A</sub> receptors is an attractive approach for the development of putative cognition-enhancing compounds, which are still far from clinical application. Several ligands with binding and/or functional selectivity for  $\alpha_5$  GABA<sub>A</sub> receptors have been synthesized and tested in a few animal models. PWZ-029 is an  $\alpha_5$  GABA<sub>A</sub> selective inverse agonist whose memory enhancing effects were demonstrated in the passive avoidance task in rats and in Pavlovian fear conditioning in mice. In the present study we investigated the effects of PWZ-029 administration in novel object recognition test and Morris water maze, in normal and scopolamine-treated rats. All the three doses of PWZ-029 (2, 5 and 10 mg/kg) improved object recognition after the 24-h delay period, as shown by significant differences between the exploration times of the novel and old object, and the respective discrimination indices. PWZ-029 (2 mg/kg) also successfully reversed the 0.3 mg/kg scopolamine-induced deficit in recognition memory after the 1-h delay. In the Morris water maze test, PWZ-029 (5, 10 and 15 mg/kg) did not significantly influence swim patterns, either during five acquisition days or during the treatment-free probe trial. PWZ-029 (2, 5 and 10 mg/kg) also proved to be ineffective in the reversal of the 1 mg/kg scopolamine-induced memory impairment in the water maze. The present mixed results encourage use of a variety of tests and experimental conditions in order to increase the predictability of preclinical testing of selective  $\alpha_5$  GABA<sub>A</sub> inverse agonists.

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### 1. Introduction

Classical benzodiazepines are positive allosteric modulators at the benzodiazepine binding site of inhibitory GABA<sub>A</sub> receptors and have been widely used for half a century as anxiolytics, hypnotics, myorelaxant and anticonvulsant drugs. Soon after introduction into

clinical practice, their propensity to disrupt memory formation was recognized and became the subject of thorough examination [1,2]. Many studies examining the nature and mechanisms of such memory impairment eventually led to a discovery that negative modulation at the same receptors could elicit an opposite effect [3]. Negative modulators (i.e. inverse agonists) of the benzodiazepine binding site decrease chloride flux through the GABA<sub>A</sub> receptor ion channel, resulting in a decrease of GABAergic inhibition and increase of neuronal excitability. These molecular and neuronal mechanisms of inverse agonism are reflected

\* Corresponding author. Tel.: +381 11 3951280; fax: +381 11 3972840.

E-mail address: [miroslav@pharmacy.bg.ac.rs](mailto:miroslav@pharmacy.bg.ac.rs) (M.M. Savić).

behaviorally in increased vigilance, but also anxiogenic and proconvulsant actions [4]. Hence, inverse agonists at the benzodiazepine binding site were shown to concomitantly improve certain forms of memory in animals [3,5] and in human volunteers [6] and elicit convulsant/proconvulsant and anxiogenic effects [7,8].

In the last two decades, genetic and pharmacological studies, by the means of generation of mutant mouse lines [9] and synthesis of novel, subtype-selective ligands, respectively, have helped in linking the particular behavioral response to a distinct GABA<sub>A</sub> receptor subtype [11–13]. Of the four GABA<sub>A</sub> receptor populations ( $\alpha_1$ -,  $\alpha_2$ -,  $\alpha_3$ - and  $\alpha_5$ -GABA<sub>A</sub> receptors) that are sensitive to benzodiazepine modulation, those receptors containing  $\alpha_1$  and  $\alpha_5$  subunits were shown to be substantially involved in mediating their memory effects [10,14–17]. While the reduced expression of  $\alpha_5$  GABA<sub>A</sub> receptors has been selectively linked to facilitation of some aspects of cognition [14,15], inverse agonism at the  $\alpha_1$  subunit was also connected with the emergence of proconvulsive and convulsive activity [18,19], and hence GABA<sub>A</sub> receptors containing the  $\alpha_5$  subunit became an ultimate target for the development of putative cognition-enhancing compounds [20].

Over the years, a number of compounds with binding and/or functional selectivity for  $\alpha_5$  GABA<sub>A</sub> receptors have been synthesized and tested in different animal models and species [21–24]. Most of them improved performance in memory tasks, whilst at the same time being devoid of CNS-mediated adverse effects [25–29]. In healthy humans, pre-treatment with an  $\alpha_5$  efficacy-selective inverse agonist significantly reduced the amnesic effect of alcohol on learning a word list, thus providing a valuable proof of the concept that  $\alpha_5$  GABA<sub>A</sub> receptors play a principal role in memory processes [30]. However, to date there are no GABA<sub>A</sub> receptor inverse agonists in the clinical practice, which indicates the need for further search for ligands with optimal efficacy/safety profile, that would translate into drugs with a clear-cut cognition enhancing potential in humans [31].

PWZ-029 is a partial inverse agonist that binds with preferential affinity to  $\alpha_5$  GABA<sub>A</sub> receptors, while at  $\alpha_1$ - and  $\alpha_3$ -containing receptors exhibits weak agonistic activity. In a previous study, PWZ-029 improved passive but not active avoidance learning task in rats, showed no effect on anxiety or muscle tone, and had no convulsive activity in the tested dose range (2–20 mg/kg) [23]. In mice, PWZ-029 at a dose of 10 mg/kg was able to robustly attenuate scopolamine-induced impairment of Pavlovian fear conditioned contextual memory [32]. The aim of the present study was to test PWZ-029 in unimpaired animals as well as against a scopolamine-induced deficit in Morris water maze (MWM) and novel object recognition test (NORT), two paradigms widely used for behavioral screening of putative memory enhancing compounds [33,34].

## 2. Material and methods

### 2.1. Animals

Male Wistar rats, weighing 200–230 g, were supplied by Military Farm, Belgrade, Serbia. Rats were housed in groups of four and were maintained under standard laboratory conditions (21 ± 2 °C, relative humidity 40–45%) with free access to pellet food and tap water. They were kept on 12:12 h light/dark cycle with lights on at 07.00 h. All handling and testing took place during the light phase of the diurnal cycle. Experiments were carried out in accordance with the EEC Directive 86/609 and were approved by the Ethical Committee on Animal Experimentation of the Faculty of Pharmacy in Belgrade. Each of four experiments was performed using separate sets of experimentally naïve animals.

### 2.2. Drugs

PWZ-029, an  $\alpha_5$  selective inverse agonist, was synthesized at the Department of Chemistry and Biochemistry, University of Wisconsin–Milwaukee, USA. The compound was suspended in a vehicle containing 85% distilled water, 14% propylene glycol and 1% Tween-80, and administered intraperitoneally in a volume of 1 ml/kg, 20 minutes before the testing. Scopolamine hydrobromide (Sigma–Aldrich,

Germany) was dissolved in saline and administered intraperitoneally in a volume of 1 ml/kg, 30 min before the testing.

### 2.3. Novel object recognition test

#### 2.3.1. Apparatus and procedure

Novel object recognition test was performed as described elsewhere [35]. The apparatus consisted of a rectangular chamber (65 cm × 45 cm × 45 cm) placed in a room with a red dim illumination (~20lx). Animals were habituated to the empty chamber for 10 min, 24 h before the beginning of experiment. Novel object recognition test was divided in two phases, familiarization and testing. In the familiarization phase two identical objects were placed 15 cm from the sides of the chamber and 25 cm apart. After receiving appropriate treatment, each rat was placed in the arena between the two objects facing the opposite wall and allowed to explore them for 5 min. Animal was then removed from the apparatus and returned to the cage (1 h) or to the colony (24 h) for the prescribed retention interval. To test for object recognition, one of the familiar sample objects was replaced with a novel object and animal was returned to the apparatus and allowed to explore for 3 min. Object exploration was recorded when the animal was in close proximity (2 cm or less) and oriented to the object, and if the animal sniffed or pawed the object. Object set consisted of a semi-transparent pyramid (base 8 cm × 8 cm, height 7 cm) and a nontransparent dome-shaped paperweight (base diameter 9 cm, height 10 cm), which could not be displaced by rats. Each object was available in triplicate and all combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects [36]. After each trial, the apparatus and objects were cleaned with 70% ethanol to reduce olfactory cues.

#### 2.3.2. Experiments

In the first experiment, four groups of rats ( $n = 10$ ) received vehicle or PWZ-029 (2, 5 or 10 mg/kg) 20 min before familiarization phase. After 24 h, object recognition memory was tested. In the second experiment we examined the influence of PWZ-029 (2, 5 or 10 mg/kg) on the scopolamine-induced memory impairment in NORT. The dose of scopolamine (0.3 mg/kg) was selected according to our preliminary testing (unpublished data). There were five group of rats ( $n = 12$ –15) and each group received one of the following treatments before the familiarization phase: saline + vehicle, scopolamine (0.3 mg/kg) + vehicle and scopolamine in combination with PWZ-029 (2, 5 and 10 mg/kg). Retention interval was 1 h. Rats were monitored using the Any-Maze video-tracking software. The amount of time exploring familiar (T1) and novel (T2) objects was automatically scored, and then total time exploring (T1 + T2) and discrimination index ((T2–T1)/(T1 + T2)) were calculated.

### 2.4. Morris water maze

#### 2.4.1. Apparatus and procedure

Experiments were performed in a 2 m diameter circular pool filled to a height of 30 cm with water at 23 °C. The escape platform (15 cm × 10 cm) of the same color as a pool was submerged 2 cm below the water surface in the northeastern quadrant (NE region) of the pool. An indirect illumination in the experimental room was provided by white neon tubes fixed on the walls and many distal cues were present (doors, pipes on the walls and the ceiling, cupboards, a camera suspended above the center of the maze). On each of the five consecutive days rats were given one swimming block, consisting of two or four trials, depending on the experimental condition. Each trial lasted a maximum time of 120 s, after which rat was manually guided to the platform and left there for 15 s. Between two consecutive trials, rats were returned to their cage and given a 60-s rest. Start positions varied pseudo-randomly among the four cardinal points (W, NW, S, SE). During the acquisition phase, treatments were applied once daily before the swimming block. On the sixth day, rats were given a treatment-free probe trial (60 s) without the platform [37].

#### 2.4.2. Experiments

In the first experiment, we examined the influence of PWZ-029 administration on water maze performance in rats. Four groups of rats ( $n = 7$ ) received vehicle or PWZ-029 (5, 10 or 15 mg/kg). We used a two-trial per day protocol which is associated with diminished performance of control animals, thus allowing for potential enhancement effects to be revealed [38]. In the second experiment, a standard four-trial per day protocol was used and the influence of PWZ-029 on scopolamine-induced impairment of water maze performance was assessed. The dose of scopolamine (1 mg/kg) was selected based on our pilot data as the dose that completely disrupted acquisition in MWM. The animals were divided into five groups ( $n = 8$ ) and received one of the following treatments: saline + vehicle, scopolamine (1 mg/kg) + vehicle, and combination of scopolamine (1 mg/kg) and PWZ-029 (2, 5 and 10 mg/kg).

Animals were monitored with the Any-Maze (Stoelting, Wood Dale, USA) video analysis system. The pool was virtually divided into four quadrants, three concentric annuli and a target region consisting of the intersection of the platform quadrant and the platform (middle) annulus, as represented in an earlier paper [17]. Dependent variables chosen for tracking during the acquisition trials were: escape latency (s), path efficiency (the ratio of the shortest possible path length to actual path length), total distance travelled (m), distance in the peripheral ring (%) and swim speed

(m/s). The indices of memory, assessed during the probe trial, included time in the NE region (s), time in the target zone (s) and time in the peripheral ring (s).

### 2.5. Statistics

All numerical data presented in the figures are given as the mean  $\pm$  S.E.M. The data from the acquisition days in the Morris water were averaged for each rat (total data/total number of trials per day) and analyzed using two-way ANOVA with repeated measures. If the interaction between the day and treatment was significant, then one-way ANOVA for the treatment effect for each day was performed. The data from the probe trial were assessed using one-way ANOVA. Post hoc comparisons, where applicable, were performed using Student–Newman–Keuls (SNK) test. In NORT, total exploration times of different treatment groups were analyzed using one-way ANOVA with post hoc SNK test. The differences between exploration times spent with old and novel objects for each group were evaluated using paired *t*-test. Discrimination index was assessed by one-sample *t*-test per treatment group. Statistical analysis was performed using SigmaPlot 11 (Systat Software Inc., Richmond, USA) software. Differences were considered to be statistically significant when *p* was less than 0.05.

## 3. Results

### 3.1. Novel object recognition test

The effects of PWZ-029 administration on the rats' behavior in the novel object recognition test are depicted in Fig. 1. One-way ANOVA revealed no differences between total exploration times among different treatment groups ( $F_{(3,36)} = 0.973$ ,  $p = 0.416$ ) (Fig. 1a). However, rats receiving 2, 5 and 10 mg/kg PWZ-029 spent significantly more time exploring the new object than the familiar one ( $t = -5.884$ ,  $p < 0.001$ ;  $t = -2.712$ ,  $p = 0.024$ ;  $t = -3.526$ ,  $p = 0.006$ , respectively), whereas rats treated with vehicle showed no significant difference ( $t = -1.264$ ,  $p = 0.238$ ) in the exploration time of the familiar object compared with that of the novel one (Fig. 1b). Discrimination indices (Fig. 1c) were significantly different from zero (i.e. chance level) for the rats treated with 2 mg/kg PWZ-029 ( $t = 4.625$ ;  $p = 0.001$ ), 5 mg/kg PWZ-029 ( $t = 3.974$ ;  $p = 0.003$ ) and 10 mg/kg PWZ-029 ( $t = 3.670$ ;  $p = 0.005$ ), but not for the control group ( $t = 1.489$ ;  $p = 0.171$ ).

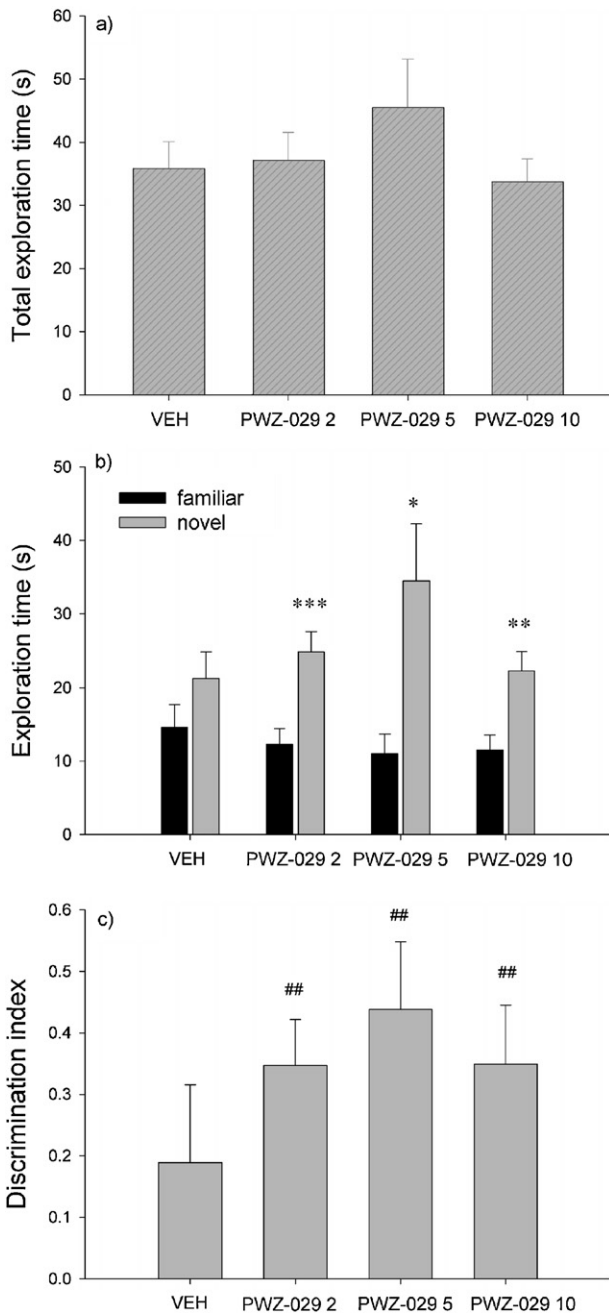
Fig. 2 shows effects of PWZ-029 administration on scopolamine-pretreated rats in NORT. Analysis of variance showed significant effect of treatment on the total exploration time ( $F_{(4,65)} = 3.167$ ,  $p = 0.019$ ); post hoc SNK test revealed that rats injected with combination of 0.3 mg/kg scopolamine and 5 mg/kg PWZ-029 spent less time ( $p = 0.017$ ) in exploring behavior compared to the control group as well as to group that received 0.3 mg/kg scopolamine + vehicle ( $p = 0.03$ ) (Fig. 2a). Comparison of the times spent in exploring familiar and novel object showed that only control group and group treated with combination of 0.3 mg/kg scopolamine and 2 mg/kg PWZ-029 spent significantly more time in exploring new object ( $t = -2.276$ ,  $p = 0.044$ ;  $t = -3.106$ ,  $p = 0.008$ , respectively) (Fig. 2b). Similarly, discrimination indices (Fig. 2c) were significantly different from zero for the control group ( $t = 2.522$ ;  $p = 0.028$ ) and group treated with combination of 0.3 mg/kg scopolamine and 2 mg/kg PWZ-029 ( $t = 2.552$ ;  $p = 0.023$ ).

### 3.2. Morris water maze

The application of PWZ-029 during acquisition days did not significantly influence swim patterns in the Morris water maze (Table 1). None of the doses tested (5, 10 and 15 mg/kg) significantly influenced any of the four indices of memory during training days: escape latency (Fig. 3a), path efficiency (Fig. 3b), total distance travelled (Fig. 3c) and percent of the distance in the peripheral ring (Fig. 3d). This reflected in performance during probe trial, where no significant differences were found among treatments in the time spent in the NE region (Fig. 4a), time in the target zone (Fig. 4a) and time in the peripheral ring (Fig. 4b).

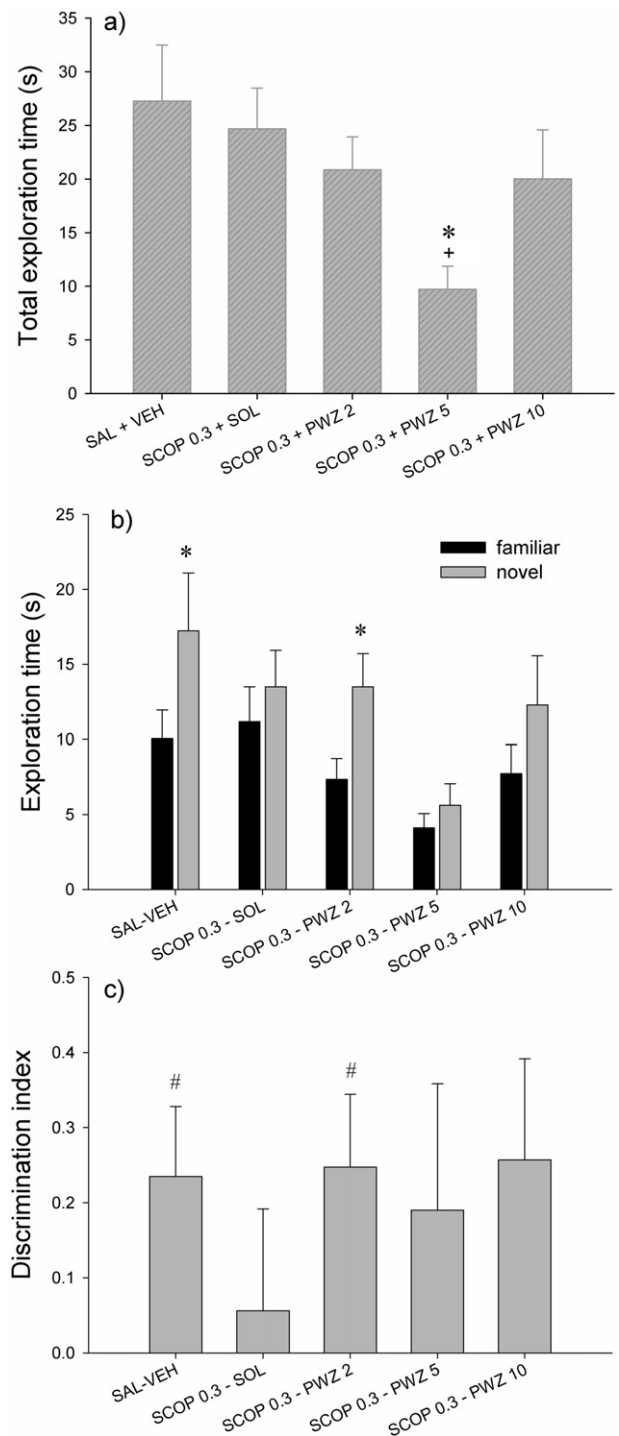
**Table 1**  
The influence of factors treatment and day on different parameters of MWM performance during acquisition days, and respective *F* and *p* values from the two-way ANOVAs with repeated measures.

Experiment parameter	VEH, PWZ-029 (5, 10 and 15 mg/kg)			SAL + VEH; SCOP 1 + VEH; SCOP 1 + PWZ-029 (2, 5 and 10 mg/kg)		
	Treatment	Day	Interaction	Treatment	Day	Interaction
Escape latency (s)	$F_{(3,24)} = 0.733$ $p = 0.542$	$F_{(4,96)} = 26.289$ $p < 0.001$	$F_{(12,96)} = 1.064$ $p = 0.399$	$F_{(4,35)} = 4.324$ $p = 0.006$	$F_{(4,140)} = 62.947$ $p < 0.001$	$F_{(16,140)} = 1.553$ $p = 0.090$
Path efficiency	$F_{(3,24)} = 0.121$ $p = 0.947$	$F_{(4,96)} = 11.296$ $p < 0.001$	$F_{(12,96)} = 1.00$ $p = 0.455$	$F_{(4,35)} = 8.355$ $p < 0.001$	$F_{(4,140)} = 14.637$ $p < 0.001$	$F_{(16,140)} = 1.49$ $p = 0.112$
Total distance (m)	$F_{(3,24)} = 1.799$ $p = 0.174$	$F_{(4,96)} = 19.234$ $p < 0.001$	$F_{(12,96)} = 1.076$ $p = 0.389$	$F_{(4,35)} = 5.355$ $p = 0.002$	$F_{(4,140)} = 35.616$ $p < 0.001$	$F_{(16,140)} = 2.392$ $p = 0.003$
Distance in the peripheral ring (%)	$F_{(3,24)} = 1.365$ $p = 0.277$	$F_{(4,96)} = 26.638$ $p < 0.001$	$F_{(12,96)} = 0.516$ $p = 0.900$	$F_{(4,35)} = 2.744$ $p = 0.044$	$F_{(4,140)} = 76.725$ $p < 0.001$	$F_{(16,140)} = 1.122$ $p = 0.341$



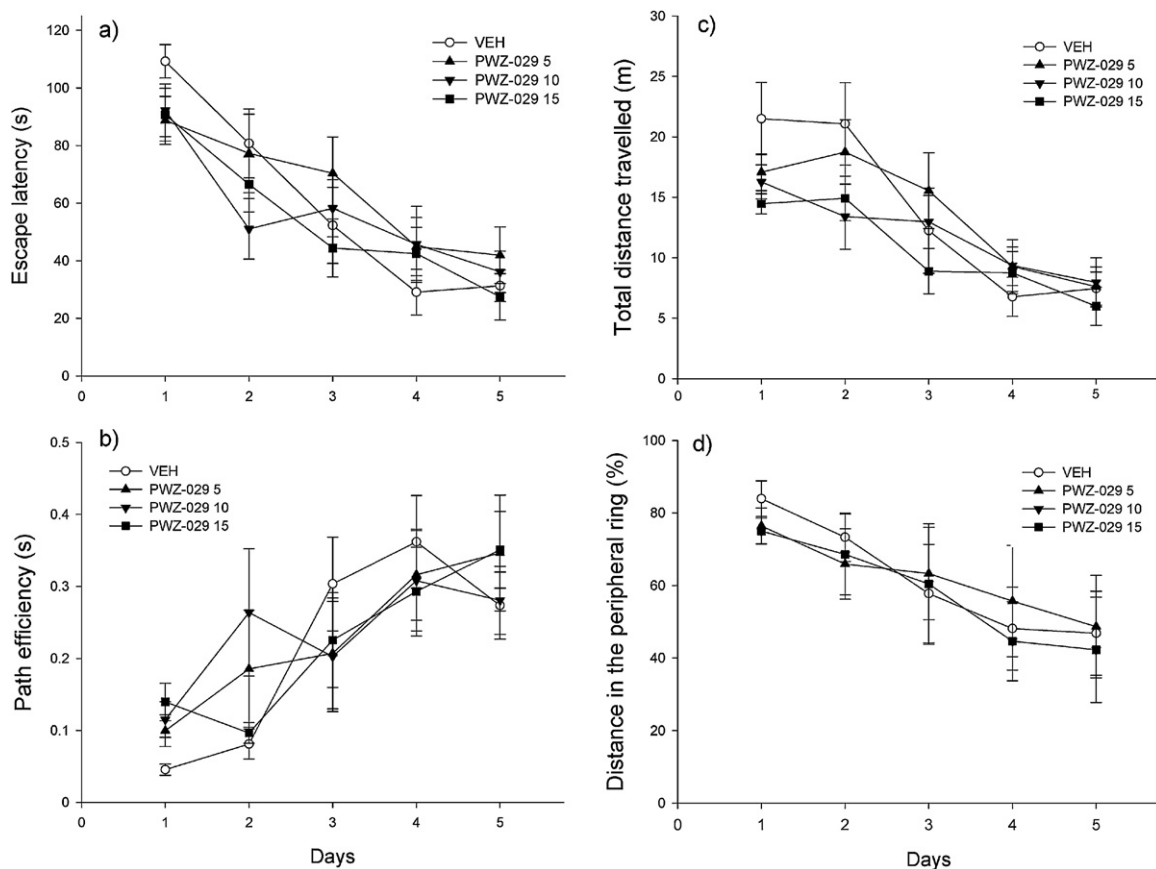
**Fig. 1.** The effects of PWZ-029 (2, 5 and 10 mg/kg) on (a) total exploration time, (b) time exploring familiar and novel objects and (c) discrimination indices in the novel object recognition test using a 24-h delay (mean + SEM). Significant differences from control (VEH) are indicated with asterisks (paired-samples *t*-test, \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001). A significant difference from zero is indicated with hashes (one sample *t*-test, ##*p* < 0.01). Number of animals per each treatment group was 10. VEH = vehicle.

In the second experiment, there were significant effects of factor treatment and factor day regarding all the parameters observed during acquisition (Table 1). After the post hoc analysis was performed, it appeared that all groups treated with scopolamine (scopolamine in combination with vehicle or 2, 5 and 10 mg/kg PWZ-029) increased escape latency compared to control (respective *p* values from SNK test: 0.009, 0.006, 0.019 and 0.019), and had lower path efficiency (respective *p* values: <0.001, <0.001, <0.001 and *p* = 0.002). The respective significant differences among treatments during days are presented in Fig. 5a and b. Scopolamine pretreatment was also a source of significant variation among



**Fig. 2.** The effects of 0.3 mg/kg scopolamine (SCOP 0.3) and combination of 0.3 mg/kg scopolamine and PWZ-029 (2, 5 and 10 mg/kg) on the rats' performance in the object recognition task after a 1-h delay: (a) total exploration time, (b) time exploring familiar and novel objects and (c) discrimination index. Data are represented as mean + S.E.M. Significant differences from control (SAL + VEH) are indicated with asterisks (\**p* < 0.05 for one-way ANOVA on total exploration time with post hoc SNK, and for paired-samples *t*-test for the familiar vs. novel exploration times); \**p* < 0.05 significant difference from SOL + SCOP 0.3. A significant difference from zero is indicated with hashes (one sample *t*-test, #*p* < 0.05). Number of animals per each treatment group was 12–15. SAL = saline, VEH = vehicle.

groups regarding the total distance travelled (Fig. 5c) and % of distance in the peripheral ring (Fig. 5d). Scopolamine increased total distance compared to control (*p* = 0.005), and addition of PWZ-029 did not influence this score since the groups treated with



**Fig. 3.** The influence of PWZ-029 (5, 10 and 15 mg/kg) on parameters related to learning in the 2-trial per day version of Morris water maze test: (a) escape latency, (b) path efficiency, (c) total distance travelled and (d) % of distance in the peripheral ring (mean  $\pm$  SEM). Number of animals per each treatment group was 7. VEH = vehicle.

combination of scopolamine and PWZ-029 (2, 5 and 10 mg/kg) still had significantly longer swim path than control group (respective  $p$  values: 0.007, 0.002 and 0.005). Additionally, all scopolamine-treated groups swam significantly faster during acquisition days (data not shown).

In Fig. 6a, the time that rats spent in the platform quadrant (NE) during probe trial is presented alongside the respective time in the portion of NE quadrant lying in the platform annulus of the maze ('the target region'). There were no significant differences among groups regarding time spent in the NE quadrant. However, there was a significant effect of treatment for the time spent in the target region ( $F_{(4, 35)} = 2.682$ ,  $p = 0.047$ ). Post hoc test revealed that rats treated with combination of scopolamine and 5 mg/kg PWZ-029 spent significantly less time in the target region ( $p = 0.043$ ). During probe trial, groups also spent significantly different times in the peripheral ring ( $F_{(4,35)} = 5.137$ ,  $p = 0.002$ ). Rats treated with scopolamine and rats treated with combination of scopolamine and PWZ-029 (2, 5 and 10 mg/kg) spent more time searching in the peripheral ring compared to control. The respective differences ( $p = 0.003$ ,  $p = 0.005$ ,  $p = 0.004$  and  $pp = 0.004$ ) are presented in Fig. 6b.

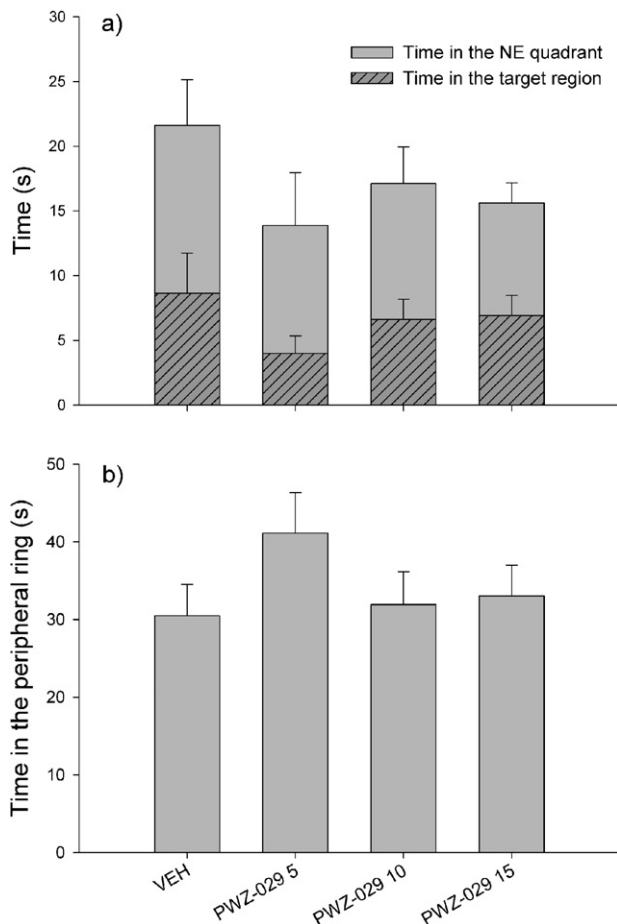
#### 4. Discussion

A substantial amount of preclinical research has focused on identifying compounds that may improve different aspects of cognition. Successful translation from promising in vitro properties into a clinically efficacious and safe therapy has long been recognized as a major bottle-neck in this field, which necessitates screening of newer ligands and thorough assessment of their effects

in a diversity of the behavioral tasks in normal and experimentally impaired laboratory animals [31,39].

PWZ-029 is one of the several inverse agonists whose selective affinities and/or efficacies at  $\alpha_5$  GABA<sub>A</sub> receptor population were set as a basic premise for their protective or therapeutic action in various cognitive impairments, and whose positive results in preclinical studies have already been published [40]. To date, memory enhancing effects of PWZ-029 were demonstrated in the passive avoidance task in rats and in Pavlovian fear conditioning test in mice. At the same time, PWZ-029 proved to be devoid of serious adverse effects such as anxiogenic and convulsive activity [23,32]. In the present study, PWZ-029 was tested in normal and scopolamine-impaired rats using two paradigms indicative of different forms of memory, the novel object recognition test and Morris water maze.

Object recognition test examines capability of animals to spontaneously discriminate between familiar and novel objects after the prescribed retention interval. Normal rats accurately discriminate between the novel and the familiar object after a 1-h but usually not after a 24-h delay [41]. The delay-dependent decline in memory recognition is believed to be a result of failure of the perirhinal cortex to maintain information about the object during longer retention intervals [42,43]. In the present experiment, PWZ-029 was able to improve object recognition after a 24-h delay, which is shown by the difference between exploration time of novel and old objects as well as discrimination indices. The result is complementary to findings that both systemic and central (in the perirhinal cortex) administration of benzodiazepines produced impairment in object recognition task, indicating involvement of GABAergic neurotransmission in regulation of this form of learning [44–46]. Since the cited studies used nonselective agonists of the benzodiazepine



**Fig. 4.** Parameters of memory retention assessed during the drug-free probe trial, in animals previously treated with 5, 10 and 15 mg/kg PWZ-029: (a) time in the NE quadrant and time in the target region (striped bars) and (b) time in the peripheral ring. Data are represented as mean + S.E.M. Number of animals per treatment group was 7. VEH = vehicle.

binding site, it remained unclear whether the impairment occurred due to activity at widespread  $\alpha_1$  subunit-containing receptors or at  $\alpha_5$  GABA receptors, whose distribution is limited to the hippocampus and certain cortical structures [47]. Results from our study showed for the first time that memory enhancement in the object recognition task can be achieved by systemic administration of an  $\alpha_5$  GABA selective inverse agonist to unimpaired rats, suggesting the significance of  $\alpha_5$  GABA receptor population in this type of memory. However, in order to confirm the present results, additional experiments using full agonists and antagonists selective for the  $\alpha_5$  subunit would be worthwhile.

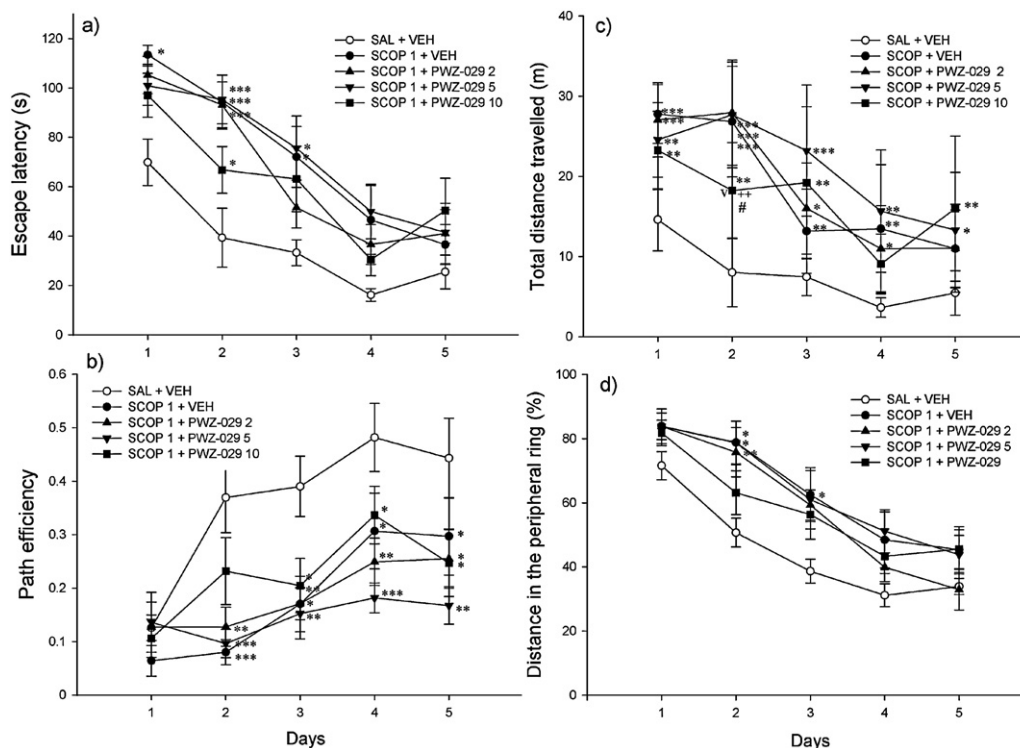
Scopolamine-induced cognitive impairment is frequently used to model the memory, attention, and executive function-related deficits that are seen in aging and dementia [48]. In the NORT, rats treated with scopolamine do not discriminate between the familiar and the novel object after a 1-h delay. The hypothesis that negative modulation of GABA<sub>A</sub> receptors could facilitate cholinergic transmission and thus improve cognitive functions is based on the fact that cholinergic pathways in certain brain regions involved in learning/memory processes are under the strong inhibitory influence of GABA neurons [49,50]. Results from our study supported this hypothesis as PWZ-029 successfully reversed the scopolamine-induced deficit in recognition memory after a 1-h delay. However, only the lowest dose of PWZ-029 (2 mg/kg) was capable of shifting the difference in time exploring novel vs. familiar objects and discrimination index to the control levels. The inefficacy of the two

higher PWZ-029 doses (5 and 10 mg/kg) in this model could be discussed from the point of possible nonspecific behavioral effects of the combined treatment. For example, combination of scopolamine and 5 mg/kg PWZ-029, but neither of these treatments alone, resulted in a significant decrease of the total exploration time, which may have confounded the results.

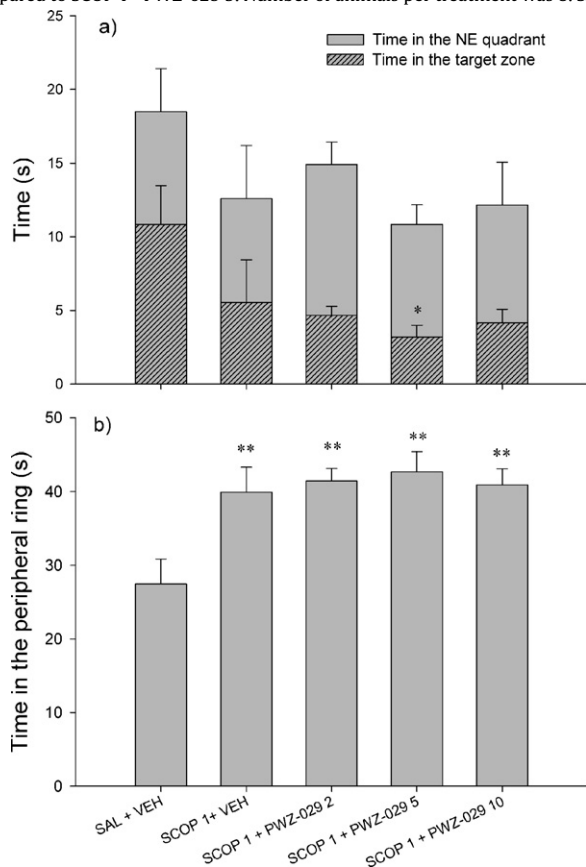
Unlike NORT which measures cognitive capability, MWM is a paradigm which tests cognitive efficiency, i.e. it tests whether treatment groups significantly differ from control in efficiency to find a hidden platform (cf. Ref. [31]). In a delayed matching-to-position version of MWM task, which tests spatial working memory (WM) when the platform position changes every day, this difference is revealed through the saving times between the first and the second trial on each of the testing days. In this version of the task, mice lacking the  $\alpha_5$  GABA<sub>A</sub> receptor subunit [14], as well as rats treated with inverse agonists at  $\alpha_5$  GABA<sub>A</sub> receptors [27], have been reported to have greater saving times than control. In the protocol that tests reference memory (RM), however, position of the platform remains the same throughout the days, and latency, distance and other related parameters are calculated. In RM protocol, McNamara and Skelton [38] reported improvement with the nonselective GABA<sub>A</sub> inverse agonist  $\beta$ -CCM. Nonetheless, rats treated with PWZ-029 remained at the control level in the same protocol. The lack of efficacy of an  $\alpha_5$  GABA<sub>A</sub> receptor inverse agonist in the current experiment could be considered in the light of the relative importance of the  $\alpha_5$  and  $\alpha_1$  modulation in the RM protocol. Our previous study [17] suggested a greater contribution of the  $\alpha_1$  than the  $\alpha_5$  subunit-containing GABA<sub>A</sub> receptors to the diazepam-induced impairment in RM protocol, which clearly reduced probability of detecting putative facilitating effects exerted via inverse agonism at the  $\alpha_5$  subtype. Furthermore, although both WM and RM protocols measure spatial memory, they reflect distinct mnemonic processes, which probably have distinct neurobiological mechanisms and thus are susceptible to modulation to a different degree [51,52]. Since there are no other reports on the use of a selective  $\alpha_5$  GABA<sub>A</sub> receptor inverse agonist in unimpaired animals in RM protocol, the suggested explanation needs to be confirmed by further research.

There are numerous examples where administration of a cognition-enhancing drug exerted no measurable effects in normal animals, whereas improved the measured cognitive parameters in subjects with experimentally induced deficits (reviewed in Ref. [39]). In our study, however, this was not the case, since PWZ-029 failed to reverse the scopolamine-induced impairment in MWM. Hence, besides the previously shown lack of activity of PWZ-029 on its own in RM protocol, an overall inefficiency of PWZ-029 in this experimental model may be postulated. In a similar experimental setup, it was shown that both, S8510 and CGS8216, an inverse agonist and an antagonist at GABA<sub>A</sub> receptors, reversed the scopolamine-induced deficit in MWM [53]. However, the other nonselective inverse agonist, FG7142, that had been previously shown to improve short-term memory in unimpaired rats [54], was ineffective in this manner [53]. Taken together, these examples with MWM results imply a limited and substance- and task-dependent potential of efficacy of inverse agonists at GABA<sub>A</sub> receptors.

In conclusion, our study showed for the first time that an inverse agonist at  $\alpha_5$  GABA<sub>A</sub> receptors, PWZ-029, may be efficacious in improving performance in the novel object recognition test in unimpaired rats, and also ameliorates deficits in memory and learning function induced by a muscarinic antagonist in this test. On the other hand, PWZ-029 proved to be inefficacious in Morris water maze, either alone or in reversing the scopolamine-induced impairment. These results indicate that behavioral screening of the putative cognition-enhancing compounds should include a



**Fig. 5.** The effects of 1 mg/kg scopolamine (SCOP 1 + VEH) and combination of 1 mg/kg scopolamine and 2, 5 and 10 mg/kg PWZ-029 (SCOP 1 + PWZ-029 2, SCOP 1 + PWZ-029 5 and SCOP 1 + PWZ-029 10) on: (a) escape latency, (b) path efficiency, (c) total distance travelled and (d) % of distance in the peripheral ring during 5-day acquisition trials in the water maze. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , compared to control (SAL + VEH); \*\* $p < 0.01$  compared to SCOP 1 + VEH, # $p < 0.05$  compared to SCOP 1 + PWZ-029 2,  $\vee p < 0.05$  compared to SCOP 1 + PWZ-029 5. Number of animals per treatment was 8. SAL = saline, VEH = vehicle.



**Fig. 6.** The representative parameters of water-maze performance in the probe trial: (a) time spent in the NE quadrant and time spent in the target zone (stripped bars), and (b) time in the peripheral ring. Data are represented as mean + S.E.M. \* $p < 0.05$ , \*\* $p < 0.01$ , compared to control (SAL + VEH). Number of animals per treatment group was 8. SAL = saline, VEH = vehicle.

variety of tests, models and experimental conditions in order to increase the predictability of the sometimes erratic positive pre-clinical results.

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