RESEARCH PAPER

Acta Neurobiol Exp 2020, 80: 364–374
DOI: 10.21307/ane-2020-033

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Protective effects against memory impairment induced by methylglyoxal in mice co-treated with FPS-ZM1, an advanced glycation end products receptor antagonist

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Memory impairment is a feature of several diseases and detrimental as aging population have increased worldwide. Sustained advanced glycation end products (AGEs) receptor (RAGE) activation triggers the production of reactive oxygen species and inflammatory response, leading to neuronal dysfunction and neurodegenerative disorders. Methylglyoxal (MGO) is the most relevant and reactive glycating agent *in vivo*, leading to the formation of AGEs. Here, we investigated the role of RAGE on the memory impairment induced by MGO. Swiss female mice were treated for 11 days with MGO, FPS-ZM1 (a high-affinity RAGE antagonist), or the combination of both. Locomotor activity was not impaired by the treatments, as evaluated by the open field and spontaneous alternation test. MGO treatment impaired short- and long-term spatial memory in the object location task, caused deficits on the short-term aversive memory in the step-down inhibitory avoidance task, and decreased working memory performance as evaluated by the Y-maze spontaneous alternation test. FPS-ZM1 treatment abolished deficits on the short-term aversive memory and working memory, but was unable to prevent the impairment in short-term or long-term spatial memory. Since the addition of RAGE antagonist in co-treatment with MGO protected mice from the aversive and working memory deficits, AGEs generated by the MGO treatment would be involved in the memory impairment due to RAGE activation. Therefore, further studies are required to establish the involvement of RAGE in the MGO-induced memory impairment. Nevertheless, our results suggested FPS-ZM1 treatment as a promising new therapeutic strategy to prevent cognitive dysfunction caused by dicarbonyl stress, further investigation is required to confirm our findings.

Key words: methylglyoxal, RAGE, AGEs, FPS-ZM1, memory

INTRODUCTION

Aging population has increased worldwide, and cognitive dysfunction has become of increasing concern. Therefore, the development of strategies and safe treatments to mitigate cognitive dysfunction are needed (Livingston et al., 2017). Likewise, memory impairment is a feature of several metabolic, genetic and degenerative diseases. These different conditions can be comorbid, reinforcing each other in augmenting cognitive decline, and in some cases, leading to dementia (Farruggia and Small, 2019; von Arnim et al., 2019).

Methylglyoxal (MGO) is a reactive dicarbonyl generated endogenously, mainly by spontaneous dismutation of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, two intermediary metabolites of glycolytic pathway (Thornalley, 1996; 2005). The serum concentration of MGO in humans was positively associated

Received 8 March 2020, accepted 12 June 2020

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with poorer memory and executive function, and a faster rate of cognitive decline (Beeri et al., 2011; Srikanth et al., 2013). Moreover, the levels of MGO are elevated in patients with mild cognitive impairment (MCI) (Haddad et al., 2019). Also, diabetic patients and diabetic rats showed elevated MGO in the plasma (Thornalley, 1993; Huang et al., 2012; Kong et al., 2014). In this regard, about 422 million people have diabetes, with increasing numbers globally (WHO, 2020), which is allied to the fact that different studies have shown that diabetes can lead to cognitive impairment (Biessels et al., 2006; Kodl and Seaquist, 2008; Kopf and Frölich, 2009).

Over the past decade, besides diabetes (Matafome et al., 2013; Tian et al., 2014), MGO has been linked to several conditions, such as cancer (Geng et al., 2014); Alzheimer's disease (Angeloni et al., 2014); hyperalgesia and inflammation (Koivisto et al., 2014); anxiety and depression (Distler and Palmer, 2012); and epilepsy (McMurray et al., 2014). However, very little is known about the mechanisms of action of MGO in the context of learning and memory (Watanabe et al., 2014; Schalkwijk and Stehouwer, 2020).

MGO is the most relevant and reactive glycating agent *in vivo*, leading to the formation of advanced glycation end products (AGEs) (Thornalley, 2005). MGO reacts with amino groups of basic amino acids of proteins, causing a loss of positive charges and promptly leading to the formation of hydroimidazolones (Thornalley, 2008; Xue et al., 2014). Hydroimidazolone adducts generated by MGO are physiological ligands of the receptor for AGEs (RAGE) (Xue et al., 2014). Since potential anti-AGEs drugs and their mechanisms of action have not been elucidated, it highlights the importance for investigating the effects of MGO burden on RAGE modulation (Toprak and Yigitaslan, 2019).

RAGE belongs to the immunoglobulin superfamily and is expressed on the surface of astrocytes, neurons and microglia (Gonzalez-Reyes and Rubiano, 2018; Palanissami and Paul, 2018). RAGE contains an extracellular V domain that binds multiple ligands, including AGEs, beta-amyloid (Aβ) protein, and high-mobility group box-1 (HMGB1). Sustained RAGE activation by AGEs leads to reactive oxygen species (ROS) production and inflammatory response, which are associated with neuronal dysfunction and neurodegenerative disorders (Sorci et al., 2013; Cai et al., 2016; Kay et al., 2016; Palanissami and Paul, 2018). Therefore, preclinical and clinical studies have pointed out that drugs capable of blocking RAGE may be useful as therapeutic tools, able to alleviate cognitive dysfunction of Alzheimer's disease patients (Yamagishi et al., 2008; Deane et al., 2012; Cai et al., 2016; Hong et al., 2016). Moreover, memory deficits induced by the treatment with HMGB1 were prevented in RAGE knockout mice (Mazarati et al., 2011).

FPS-ZM1 is a high-affinity RAGE antagonist, permeable to the blood-brain barrier that binds to the V domain of RAGE. In rodents, FPS-ZM1 was able to inhibit RAGE-mediated influx of circulating Aβ40 and Aβ42 into the brain (Deane et al., 2012). In addition, FPS-ZM1 inhibited β-secretase activity, thus, decreasing Aβ formation, microglia activation and inflammatory responses. Nevertheless, results from different models have shown that FPS-ZM1 can be beneficial. Treatment with FPS-ZM1 was effective in normalizing cognitive performance in $APP^{sw/0}$ transgenic mouse model of Alzheimer's disease and in an AGEs-RAGE activated rat model (Deane et al., 2012; Hong et al., 2016). Also, FPS-ZM1 can decrease cell infiltration and edema in a model of brain hemorrhage, decreasing oxidative and cellular damage to the renal tubule (Deane et al., 2012; Li et al., 2015; Hong et al., 2016; Lian et al., 2017; Sanajou et al., 2019).

In a previous work, we demonstrated that repeated MGO (50 mg/kg) treatment, for 11 days by systemic injection, caused extensive memory impairment in mice (Szczepanik et al., 2020). In order to investigate whether memory impairment induced by MGO was dependent on RAGE activation, mice were treated with the RAGE antagonist FPS-ZM1 in co-administration with MGO for 11 days.

METHODS

Animals and treatments

Experiments were conducted using 3-month-old female Swiss mice (35-55 g) bred at the Federal University of Santa Catarina (UFSC), Florianópolis, Brazil. Mice were maintained in groups of 7-10 animals per cage (42 × 34 × 17 cm), under controlled temperature (22±1°C), and 12 h light cycle (lights on at 7:00 AM), with free access to food (standard chow diet) and water. Efforts were made to minimize the number of animals used and their suffering.

We used mice of the same age and sex that were investigated in our previous treatment protocol (Szczepanik et al., 2020). Older age is a primary risk factor for cognitive decline, and cognitive dysfunction in healthy young humans is a rare condition (Carpenter et al., 2019). Therefore, we aimed to investigate the MGO-induced memory impairment on the adulthood. We choose mice of 3 months old (90 days) in the beginning of the treatments (day 0). Considering life stages of *Mus musculus*, 3 months old mice over our experimental protocol of 13 days resembled equivalent to 27-32 years old in human age (Dutta and Sengupta, 2016).

Female subjects are underrepresented in pre-clinical research, particularly in the field of neuroscience

where it is found over five studies on males for each study on females (Beery, 2018). Since female rodents are not more variable than males, and, given the lack of studies using female subjects, we decided to investigate female mice, which will likely result better understanding of female, and eventually, translate to better treatment outcomes for women, as indicated by others (Prendergast et al., 2014; Beery, 2018).

MGO (pyruvaldehyde) (CAS *Number*: 78-98-8) was purchased from Sigma-Aldrich (São Paulo, Brazil), and FPS-ZM1 [4-chloro-N-cyclohexyl-N-(phenylmethyl)-benzamide] (#6237, CAS *Number*: 945714-67-0), was purchased from Tocris Bioscience (São Paulo, Brazil). Mice were treated with 0.9% saline (vehicle: control); 20 or 50 mg/kg MGO; 2 mg/kg FPS-ZM1 diluted in vehicle, or the co-administration of MGO and FPS-ZM1. The FPS-ZM1 dose chosen was based upon two previous studies (Deane et al., 2012; Lian et al., 2017). The drugs were administered by intraperitoneal injection (i.p.) with a relative injection volume of 1 ml / 100 g of body weight.

Each mouse received two i.p. injections on contralateral sides each day, during 11 days, making up the following groups: saline + saline (control), saline + MGO, FPS-ZM1 + saline, or FPS-ZM1 + MGO (N=7-10 animals per group). To evaluate the effects of repeated treatment with FPS-ZM1 and MGO, behavioral tests were performed at indicated time points, always 24 h after the last MGO injection (Fig. 1), as follows: handling (day 0); administration of MGO (days 1-11); open field (day 5); object location task (days 5-9); Y-maze spontaneous alternation (day 10); step-down inhibitory avoidance task (day 11). To avoid acute effects and possible withdrawal syndrome, mice were treated with MGO 2 h after the behavioral testing.

To test the acute effect, an independent cohort of mice received a single injection of MGO (20 mg/kg) and/or FPS-ZM1 (2 mg/kg) to investigate spatial short-term memory using the object location task.

In all treatments, the RAGE antagonist FPS-ZM1 was injected prior to MGO, following a 30 min interval between injections.

Experimental procedures

All the procedures used in this study were authorized and complied with the guidelines on the animal care of the UFSC Ethics Committee on the Use of Animals (CEUA/UFSC, protocol number: 7245210616), a local committee which follows the "ARRIVE guidelines" and the "Guide for the Care and Use of Laboratory Animals" from NIH. Behavioral tests were conducted between 09:30 and 16:30 h in a dimly lit and sound-isolated room: 15 lx for the open field and object location task; 30 lx for the remaining behavioral tests. The experiments were recorded by a video camera system and images analyzed using the ANY-maze® software (Stoelting Co., Wood Dale, IL, USA). Mice were acclimatized to the experimental room for 2 h before the beginning of the tests. Once the mouse was exposed to a session paradigm, it was not mixed with non-exposed mice on its return to the home cage. In the acute treatments, an independent group of mice was used for the object location task. The total number of mice used in the behavioral tests was 52.

Open field

The spontaneous locomotor activity and anxiety-like behavior of mice were evaluated in an open field arena. In the experiment, each animal was placed in the center of the arena to freely explore the appara-

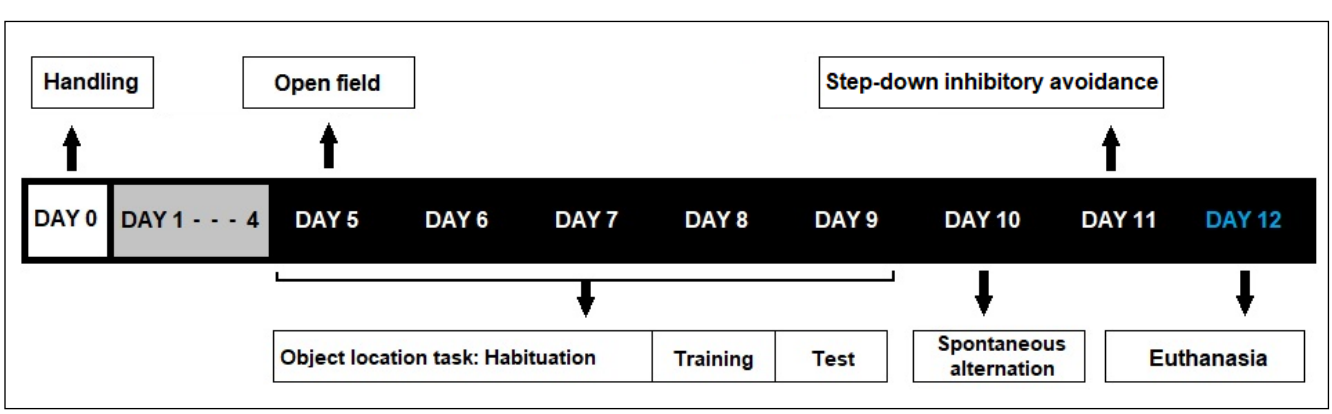

Fig. 1. Schematic representation of repeated treatment and behavioral tests. Mice were subjected to repeated treatment with saline (control), methylglyoxal (MGO), FPS-ZM1 (ZM1) or MGO + ZM1 once a day for 11 days by i.p. injection. Mice were tested in the OF (day 5), OLT (days 5-9), SA (day 10), and step-down inhibitory avoidance task (SDT, day 11).

tus for 5 min. The apparatus was made of four transparent acrylic walls (30 cm high) and gray floor of 40 cm × 40 cm. The parameters analyzed were the total distance traveled (m), average speed (m/min), the number of entries and time spent (s) by the mouse in the central area (20 × 20 cm) of the open field arena (Belzung, 1999).

Object location task

The spatial memory of mice was assessed using the object location task (OLT) to evaluate both short- and long-term memory. Evaluation of short-term memory was carried out based on a previous protocol (Assini et al., 2009). In this paradigm, each mouse was habituated in the open field arena with transparent walls for 5 min (habituation session). Twenty-four hours thereafter, mice were placed in the same arena for 5 min facing two identical objects (5 × 3 cm; training session). Ninety minutes after training, one of the objects was moved to a new location (test session), and the time spent exploring the objects in new (novel) and old (familiar) locations were recorded for 5 min. The two identical objects were placed 20 cm apart, 7 cm away from the walls, and the relative position of the relocated object was counterbalanced among the mice. Visual cues were added in the test room to serve as spatial reference. In order to analyze the cognitive performance, a location index was calculated: $(T_{novel} \times 100)/(T_{novel} + T_{familiar})$, where T_{novel} is the time spent exploring the relocated object and $T_{familiar}$ is the time spent exploring the non-relocated object (Vogel-Ciernia and Wood, 2014).

Using the above protocol, we tested an acute administration, as follows: after habituation session on the previous day, mice received an i.p. injection of 0.9% saline or FPS-ZM1 (2 mg/kg); 30 min after that, the mice received an i.p. injection of 0.9% saline or MGO (20 mg/kg), making up the following groups: saline + saline (control); saline + MGO (MGO); and FPS-ZM1 + MGO. Then, 2 h after the last injection, mice were submitted to the training session (5 min), and returned to home cage. Finally, 90 min after the training session (3.6 h after MGO administration), the test session was performed for 5 min to evaluate short-term memory.

Also, the effects of repeated treatments were evaluated 4 days after a pretreatment with MGO (50 mg/kg, i.p.), using a modified protocol to evaluate the long-term type of spatial memory. This protocol included 3 days of habituation during 5 min sessions (1 session per day), with the open field floor covered with wood shavings. This was followed by MGO administration 2 h later. On the 4th day, a training session was performed for 10 min, which was followed by MGO administration 2 h after training. The test session of 5 min was performed 24 h after the training session (Vogel-Ciernia and Wood, 2014).

Spontaneous alternation

Evaluation of working memory of mice was carried out measuring the spontaneous alternation (SA) rate in a Y-maze, which was positively related to better memory performance. When moving from one place to another, rodents exhibit the natural tendency to explore the least visited area or a previously known area which has changed (novelty), this behavior is referred to as spontaneous alternation (Tolman, 1925). Spontaneous alternation was assessed using a Y-shaped maze, made of opaque gray acrylic, with three equal arms (30 cm length, 10 cm width, 25 cm height), disposed at 120° from each other and connected by a triangular central area (neutral zone). In the test, each mouse was placed in the neutral zone and allowed to explore the maze area for 5 min. An arm entry was computed only when the mouse entered with all four paws. The total number of arm entries (N) was used as a parameter of locomotor activity. The number of 'correct' triplets (M, consecutive choices of each of the three arms without re-entries) was also registered. Based on the 'correct' triplets, the spontaneous alternation rate was computed according to the formula: $R(\%) = M \times 100/(N-2)$ and used as parameter of cognitive performance (Dember and Fowler, 1958; Kleschevnikov et al., 2017).

Step-down inhibitory avoidance task

To assess short-term aversive memory, mice were exposed to the step-down inhibitory avoidance task (SDT). The apparatus was a 23 × 21 × 21 cm^3 box made of acrylic and steel in which the floor was composed of a parallel grid of stainless-steel bars with 4 mm diameter spaced 1 cm apart. A vinyl platform (11 × 8 cm wide, 3 cm high) was placed in the center of the floor. Based on previously described procedures (Roesler et al., 1999; Moreira et al., 2012), each mouse was placed on the platform and its latency to step down on the grid with all four paws was measured. During the training session, immediately after stepping down on the grid, the mouse received a 2 s long scrambled foot shock (0.3 mA), then it was transferred to its home cage. This task is based on the mice's avoidance behavior to step down the platform after receiving the electric shock. To evaluate memory retention, a test session was performed 1.5 h after the training session. The test session was identical to the training session,

except that no foot shock was given. A maximum of 180 s per session was waited to the mouse stepping down on the grid.

Statistical analysis

Statistical analyses were performed using one-way or two-way analysis of variance (ANOVA), appropriate in each case, followed by the Newman-Keuls *post hoc* test. Additional analysis were made for the OLT using Student's t-test to compare the location index values with the random chance of 50%, related to the exploration time of the two objects in the task. In the SDT, presenting non-normal distribution, statistical analysis was performed using the Kruskal-Wallis non-parametric test followed by Dunn's comparison. The accepted level of statistical significance was $P \leq 0.05$. Data are expressed as mean ± standard error of the mean (SEM) or median (interquartile range). All statistical tests were carried out using the Statistica software package, version 7.0 (StatSoft Inc., Tulsa, OK, EUA).

RESULTS

Effects on locomotor activity

Mice were submitted to the open field on the 5th treatment day. One-way ANOVA revealed no statistically significant differences in the total distance travelled ($F_{3,18}$=0.447, P>0.05) (Fig. 2A), the average speed ($F_{3,18}$=0.447, P>0.05) (Fig. 2B), and the number of entries ($F_{3,18}$=1.057, P>0.05) (Fig. 2C) and the time spent ($F_{3,22}$=0.541, P>0.05) (Fig. 2D) in the central area of the open field apparatus.

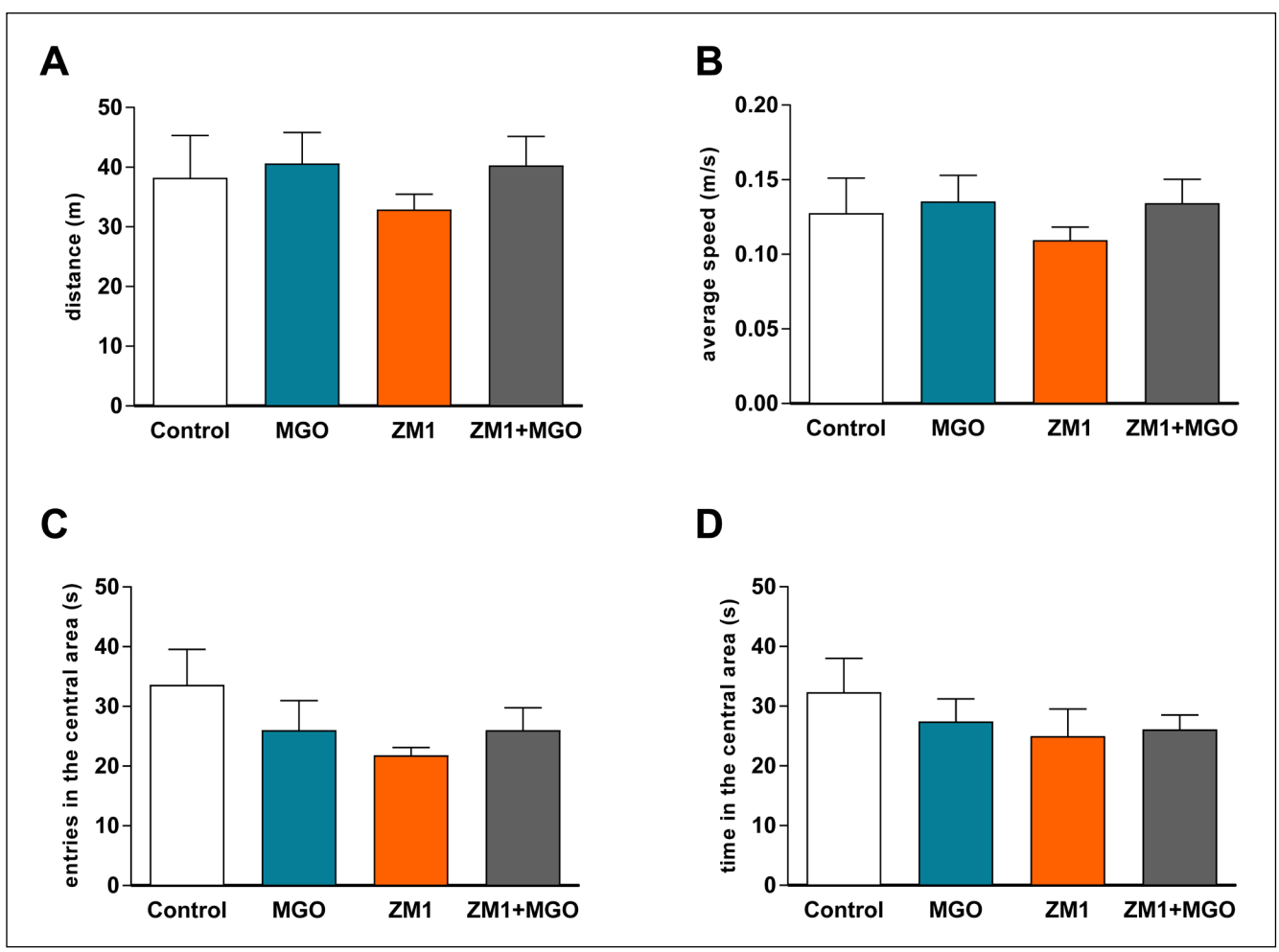

Fig. 2. Locomotor activity of mice after repeated treatment with methylglyoxal and FPS-ZM1. Mice were treated for 4 days with methylglyoxal (MGO, 50 mg/kg) or FPS-ZM1 (ZM1, 2 mg/kg) or with co-administration (ZM1 + MGO). Twenty-four hours after the last dministration, mice were evaluated for 5 min in the open field. A) Distance traveled (m); B) Average speed (m/min); C) Number of entries, and D) Time spent in the central area. The bars represent the mean ± SEM of 6-7 animals per group. No statistical differences among groups were found.

RAGE inhibitor FPS-ZM1 does not rescue spatial memory impairment induced by MGO

Mice were tested in the OLT to assess the spatial memory, on the 9th treatment day. Mice were submitted to the open field for 3 days for habituation purpose, and 24 h after the training session, long-term memory was evaluated (Fig. 3A). One-way ANOVA followed by the Newman-Keuls test revealed that the groups MGO and FPS-ZM1 + MGO showed lower location index when compared to the control group ($F_{3,24}$=7.492, P<0.05), indicating memory impairment. However, the FPS-ZM1 group showed similar location index in comparison to the control group ($F_{3,24}$=7.492, P<0.05), indicating absence of memory impairment. When the groups were compared against the random chance of exploring both objects equally (50%), results were confirmed, as well. Control (t_6=5.173, P<0.05) and FPS-ZM1 (t_6=14.57, P<0.05) groups explored the relocated object B for more than 50% of the total exploration time, thereby, showing memory retention. Mice treated with MGO (t_6=0.826, P>0.05), or FPS-ZM1 + MGO (t_6=1.059, P>0.05) did not present significant differences in the object location index, when compared to 50%, indicating memory impairment (Fig. 3A). In the training session, no differences among the groups were found between the two identical objects exploration period (Fig. 3C). Two-way ANOVA revealed no significant differences to treatment ($F_{3,48}$=1.974, P>0.05), or object factors ($F_{1,48}$=0.084, P>0.05). Also, no significant interaction between treat-

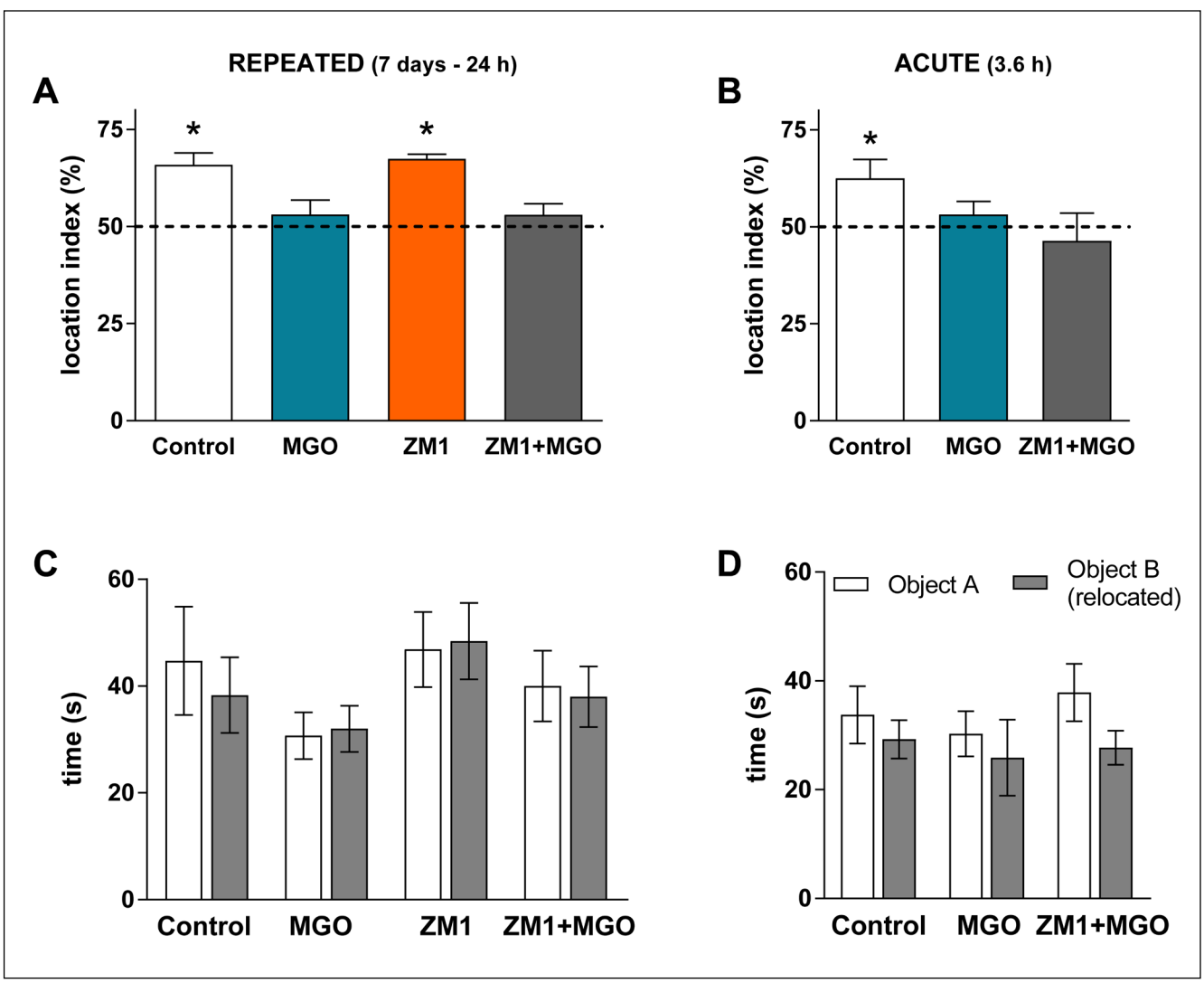

Fig. 3. Spatial memory in the OLT of mice treated with methylglyoxal and FPS-ZM1. Mice were treated for 7 days with methylglyoxal (MGO), FPS-ZM1 (ZM1, 2 mg/kg), or with both (ZM1 + MGO) and evaluated in OLT. Long term (A) or short term (B) memory was evaluated 3.6 or 24 h after the last MGO injection with 20 or 50 mg/kg, respectively. The bars represent the mean ± SEM (n=7-8 animals per group). * P<0.05 (Student's t-test, as compared to 50%; and Newman-Keuls *post hoc* test).

ment and exploration time of the two objects was revealed ($F_{3,48}$=0.151, P>0.05). Therefore, it indicates no bias due to a possible exploratory preference in the training session.

We have already demonstrated in our previous work that MGO (20 mg/kg, i.p.) induced impairment in short-term spatial memory after a single acute injection. In order to investigate whether this effect can be prevented by FPS-ZM1, an independent cohort of mice was treated once with MGO (20 mg/kg); or FPS-ZM1 (2 mg/kg) + MGO (20 mg/kg). To evaluate the short-term spatial memory, mice were tested in the OLT 90 min after the training session, and 3.6 h after MGO injection. In this case, results showed that FPS-ZM1 was also unable to prevent the impairment induced by MGO on spatial memory (Fig. 3B). One-way ANOVA followed by the Newman-Keuls test revealed that only the control group ($F_{2,19}$=2.343, P<0.05) showed significant difference on the location index, indicating memory retention. The control mice explored the object B (relocated) for a significantly longer period (t_7=2.561, P<0.05), when compared to 50%. However, mice treated with MGO (t_6=0.944, P>0.05) and FPS-ZM1 + MGO (t_6=0.503, P>0.05) showed no differences regarding a random preference of 50%, thus, indicating memory impairment. In the training sessions, two-way ANOVA revealed that the same mice explored the objects (A and B) for a similar period. No differences were found for treatment ($F_{2,42}$=0.826, P>0.05), relative exploration of the objects ($F_{1,42}$=1.732, P>0.05), or interaction between treatment and exploration time ($F_{2,42}$=0.146, P>0.05) (Fig. 3D).

RAGE inhibitor FPS-ZM1 prevent working memory deficit induced by MGO

Working memory of mice was evaluated after repeated treatment with MGO for 10 days, according to the spontaneous alternation rate, as evaluated by recording the arm entries in the Y-maze (Fig. 4). One-way ANOVA, followed by the Newman-Keuls test, revealed that mice treated with MGO showed significantly decreased alternation rate, when compared to the control, and the other groups, indicating deficit on the working memory performance ($F_{3,24}$=7.734, P<0.05). Treatment with FPS-ZM1 did not change the alternation rate in the Y-maze, as compared to the control group (P>0.05). Interestingly, results suggested that FPS-ZM1 administration prevented the working memory deficit induced by MGO, as significant difference was not observed in the FPS-ZM1 + MGO group, in comparison to the control group (P>0.05) (Fig. 4A). One-way ANOVA failed to indicate differences on the total number of arm entries ($F_{3,24}$=1.910, P>0.05), indicating locomotor activity of mice was not altered by the treatments (Fig. 4B). Also, it indicates no biases due to any locomotor effect.

RAGE inhibitor FPS-ZM1 prevent short-term aversive memory impairment induced by MGO

The SDT was used to evaluate short-term aversive memory on 11th day of treatment. Mice were tested 1.5 h after the training (learning) session, in which

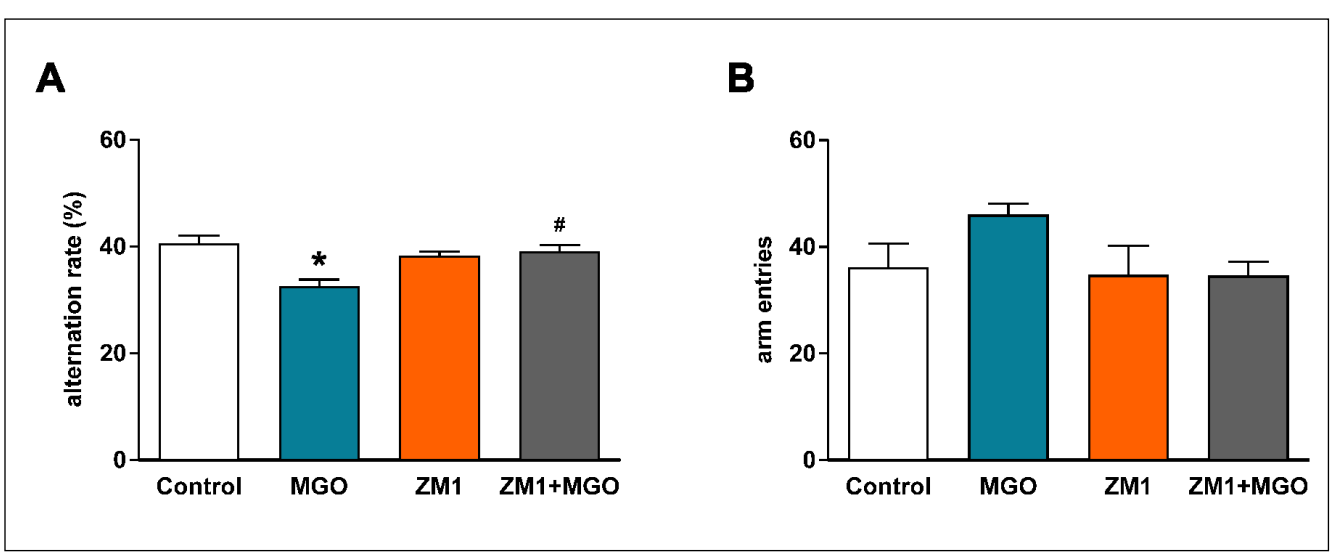

Fig. 4. FPS-ZM1 prevented working memory deficit in mice treated with MGO. Mice were treated for 9 days with methylglyoxal (MGO, 50 mg/kg), FPS-ZM1 (ZM1, 2 mg/kg), or with both (ZM1 + MGO), and 24 h after the last administration, evaluated in the Y-maze: A) Alternation rate (%), as a measure of working memory; B) Number of arm entries, as a measure of locomotor activity. The bars represent the mean ± SEM of 7 animals per group. * P<0.05 compared to the control group; # P<0.05 compared to the MGO group (Newman-Keuls *post hoc* test).

they received an electrical stimulus. The latency time to step-down from the platform was measured in both sessions. The Kruskal-Wallis analysis was significant (Chi-square=35.22, *P<0.05*), which was followed by Dunn's comparison and observed by the difference in rank sum (R diff). The control group presented a significantly longer latency time to step-down from the platform in the test session, when compared to its respective latency time in the training session (*R diff*=-23.67, *P*<0.05), indicating memory retention. However, results revealed that mice treated with MGO was unable to show increased latency time in the test session, when compared with its respective latency time in the training session (R diff=-16.50, *P*>0.05). This result indicates short-term aversive memory deficit caused by MGO. Unlike the MGO group, mice treated with FPS-ZM1 (R diff=-25.58, *P*<0.05) and FPS-ZM1 + MGO (R diff=-27.79, *P*<0.05), presented increased latency to step down, indicating memory retention. Once the FPS-ZM1 + MGO group showed increased latency time in the test session, this indicates that FPS-ZM1 was able to prevent the short-term memory impairment induced by MGO (Fig. 5).

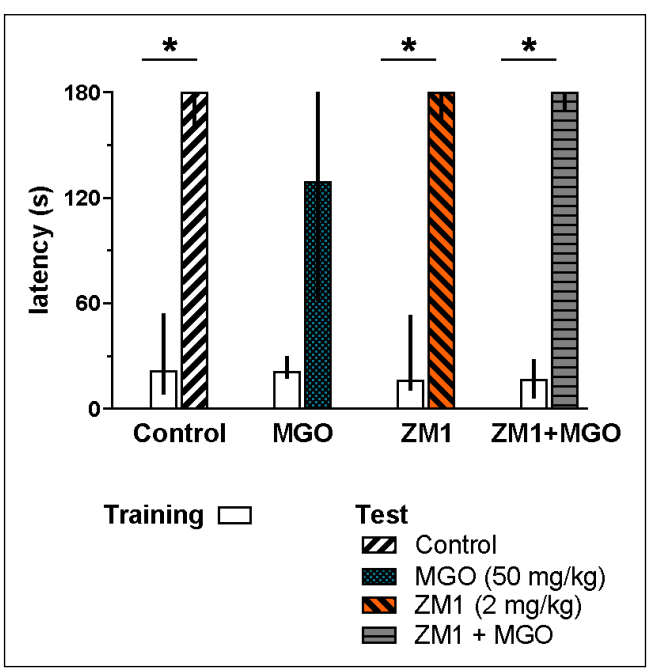

Fig. 5. FPS-ZM1 prevented the aversive memory impairment in mice treated with MGO. Mice were treated for 10 days with methylglyoxal (MGO, 50 mg/kg), FPS-ZM1 (ZM1, 2 mg/kg) or with both (ZM1 + MGO), and evaluated in the SDT, 24 h after the last MGO administration. Short-term memory was evaluated 1.5 h after the training session that consisted of an aversive electrical stimulus, but without applying this stimulus. The bars represent the median (interquartile range) of step-down latencies of 6-7 animals per group. * *P*<0.05 as compared to the training session of the same group (Kruskal-Wallis non-parametric test followed by Dunn's comparison).

DISCUSSION

RAGE effects are strongly dependent on the cell type and physiological conditions, and involved in tissue homeostasis and regeneration following injury (Sorci et al., 2013). It is already known that activation of RAGE by AGEs can lead to oxidative stress and altered gene expression (Wautier et al., 2001; Palanissami and Paul, 2018). Since MGO is the most reactive glycating agent leading to the formation of AGEs, it is thought to be a major RAGE ligand (Thornalley, 2005). Cognitive dysfunction can be triggered by ROS, inflammatory response and neuronal dysfunction after sustained RAGE activation (Cai et al., 2016; Kay et al., 2016). AGEs/RAGE signaling can be involved in the vascular complication of diabetes, leading to a variety of neuropathies, cognitive dysfunction and accelerated atherosclerosis, which could account for disabilities and high mortality rates in diabetic patients (Kodl and Seaquist, 2008; Yamagishi et al., 2008). In line with this idea, we treated young mice for 11 days with MGO and detected a clear cognitive impairment. Here, it is shown that a high-affinity RAGE antagonist (FPS-ZM1) can prevent some memory impairments induced by MGO.

Results showed that the treatments did not affect the locomotor activity or anxiety-like behavior of mice, as evaluated in the open field, OLT and SA, indicating that the memory dysfunction observed were independent of these factors. Since MGO has already been associated with locomotor depression and decreased anxiety-like behavior (Hovatta et al., 2005; Distler et al., 2012), these were relevant features to be accessed.

Evaluation of cognitive parameters showed that FPS-ZM1 alone did not cause any memory deficit. However, MGO treatment impaired short- and long-term spatial memory in the OLT, short-term aversive memory in the SDT, and decreased working memory performance of mice in the SA. All of these results corroborate our previous data obtained from the same experimental approach. Also, we observed that mice treated with MGO displayed increased depressive-like behavior, along with dopamine depletion in the cerebral cortex (Szczepanik et al., 2020). Depressive-like behavior have been associated with neuronal atrophy and synaptic loss in the cortex and hippocampus, which are neuroplasticity impairments involved in memory dysfunction (Price and Duman, 2020). Especially, depressive behaviors and anhedonia are hardly dissociated from learning and memory disorders in humans, and cognitive impairment is a hallmark of major depressive disorder (Baune and Renger, 2014). Thus, this is in line with our results from the memory tests.

Behavioral memory tests can be biased by non-mnemonic factors, such as arousal level, sensory sensitivity

and random agents (Robinson et al., 2019). In order to draw deeper conclusions from our experiments, other behavioral learning and memory tests (such as radial arm maze and Morris water maze) would be helpful. Other tests can provide complementary results regarding the degree of acquisition and expression of memory, as well as to evaluate other memory domains (Vorhees and Williams, 2006). Moreover, our 11-day treatment cannot establish the potency and duration of the effects of FPS-ZM1 or MGO, beyond the study period. Therefore, multiple trial tests, longer treatment periods and using pharmacological tools would be necessary to examine more deeply the effects of FPS-ZM1 and MGO on memory. For instance, spatial memory was not protected by FPS-ZM1 in MGO-treated mice, in acute or repeated regimen, suggesting action mechanisms other than RAGE activation would take place.

Most of the anti-RAGE antibodies available block only peripheral RAGE, and do not cross the blood-brain barrier. In addition, they may have toxic effects at therapeutic doses. On the other hand, FPS-ZM1 is permeable to the blood-brain barrier, where off target effects were evaluated in cell culture, and in mice up to a dose of 500 mg/kg (i.p.). Physiological parameters or susceptibility to infection were not altered in rodents treated with FPS-ZM1 (Deane et al., 2012). Previously, it was demonstrated that RAGE-neutralizing antibodies protected human SH-SY5Y neuroblastoma cells, and rat cortical neurons from oxidative stress and cell death induced by AGEs, in a model using glycated bovine serum aluminum (Yin et al., 2012). In the present study, we found that systemic injection of FPS-ZM1, in co-treatment with MGO, protected mice from deficits on the short-term aversive and working memory, despite its ineffectiveness toward spatial memory impairment. Based on the FPS-ZM1 positive effects on memory, we speculate that drugs, such as perindopril (increases the soluble form of RAGE) and alagebrium (cleaves AGEs cross-links), would be effective in preventing memory dysfunction. Actually, it was already shown that decreasing the AGEs/RAGE signaling can prevent working memory decline in diabetic rats, as evaluated in the Y-maze (Zakaria et al., 2015).

Activation of RAGE triggers the production of ROS via NADPH oxidase, and the activation of the transcription factor nuclear factor kappa B (NFκB), which leads to increased levels of pro-inflammatory cytokines (Sorci et al., 2013; Palanissami and Paul, 2018). Since the activation of RAGE positively modulates its own expression via NFκB responsive element (Schleicher and Friess, 2007; Fang et al., 2010), repeated MGO treatment would amplify the AGEs/RAGE signaling, potentially contributing to the cognitive impairment observed in mice. In agreement with this hypothesis, the protective effects of FPS-ZM1 against MGO-induced cognitive dysfunction can be recognized from other studies. After FPS-ZM1 treatment, streptozotocin-induced diabetic rats showed attenuated renal expressions of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Sanajou et al., 2019), two cytokines also linked to neuroinflammation (Becher et al., 2017). Moreover, FPS-ZM1 attenuated diabetes-induced elevations in renal levels of RAGE and phosphorylated NFκB p65 subunit (Sanajou et al., 2018). In aorta of aged rats, FPS-ZM1 reduced ROS and IL-6 levels, and suppressed the activation of NFκB (Gu et al., 2014). Other studies also corroborate this notion, for instance, the overexpression of microglial RAGE in transgenic mice expressing the mutant human amyloid precursor protein (mAPP), implicated in the pathogenesis of Alzheimer's disease, increased the production of pro-inflammatory mediators such as TNF-α, interleukin 1 beta (IL-1β), the infiltration of microglia and astrocytes, the accumulation of Aβ peptide, and accelerated the appearance of deficits on learning and spatial memory (Fang et al., 2010). Accordingly, the downregulation of RAGE by β-asarone was linked to memory improvement in a double transgenic mouse model of Alzheimer's disease (Yang et al., 2016). Moreover, the downregulation of both RAGE and NFκB by the *Panax ginseng* extract was associated to the protection of long-term spatial and aversive memory in a model of AGEs production in rats (Tan et al., 2015). All the findings, along with the behavioral evidences presented by this study using MGO treatment, suggest that the depression of AGEs/RAGE signaling in critical stressed tissues can result in better outcomes in cases of impaired learning and memory function.

CONCLUSIONS

Overall, our results suggest that FPS-ZM1 treatment could potentially be used to prevent cognitive dysfunction caused by dicarbonyl stress. For the establishment of FPS-ZM1 as a valid new therapeutic tool, further studies will be needed. Differences in the experimental conditions (species, behavioral tests, and dose *vs.* treatment period), could modify FPS-ZM1 effectiveness in preventing MGO-induced responses in mice. Investigating the balance between different doses of MGO and FPS-ZM1 and dose-response effects are necessary. Nevertheless, evaluating different ages, treatment regimens and long-term effects are also interesting topics for future studies. Since we used only Swiss female mice in this study, other studies using male subjects would be also necessary, as well as studying other mice strains. Thus, future studies could consolidate the ben-

eficial effects of FPS-ZM1 against the cognitive dysfunction caused by MGO.

In conclusion, results showed that MGO treatment impaired working memory, short- and long-term memory. Furthermore, data presented by this study are experimental evidence that FPS-ZM1 protected mice from the short-term aversive and working memory deficits induced by MGO. Finally, results suggested that RAGE activation was involved, at least in part, in the observed memory impairment. However, this was not the case for long-term spatial memory, indicating further studies are required to address the exact mechanisms responsible for MGO-induced cognitive dysfunction.

ACKNOWLEDGEMENTS

This work was supported by the Brazilian funding agency CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, #462333/2014-0, #306204/2014-2), and ALD is a research fellow (423644/2018-1). Fellowships were granted by CAPES (Coordination for the Improvement of Higher Education Personnel, Brazil) to MPC (post-doctoral PNPD), JCS and GRLA (doctorate), and AFG (master).

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