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The G protein-coupled estrogen receptor (GPER) regulates recognition and aversively–motivated memory in male rats

Lariza Oliveira de Souza [a], Gustavo Dalto Barroso Machado [a], Betânia Souza de Freitas [b], Sarah Luize Camargo Rodrigues [a], Maria Paula Arakaki Severo [a], Patrícia Molz [a], José Afonso Corrêa da Silva [a], Elke Bromberg [b,c], Rafael Roesler [d,e], Nadja Schröder [a,c,*]

[a] Department of Physiology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[b] Neurobiology and Developmental Biology Laboratory, School of Health and Life Sciences, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil
[c] National Institute of Science and Technology for Translational Medicine (INCT-TM), Conselho Nacional de Desenvolvimento Cientifico e Tecnologico (CNPq), Brasília, Brazil
[d] Department of Pharmacology, Institute for Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil
[e] Cancer and Neurobiology Laboratory, Experimental Research Center, Clinical Hospital (CPE-HCPA), Federal University of Rio Grande do Sul, Porto Alegre, Brazil

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ABSTRACT

Estrogens, particularly 17β-estradiol (estradiol, E_2), regulate memory formation. E2 acts through its intracellular receptors, estrogen receptors (ER) ERα and ERβ, as well as a recently identified G protein-coupled estrogen receptor (GPER). Although the effects of E2 on memory have been investigated, studies examining the effects of GPER stimulation are scarce. Selective GPER agonism improves memory in ovariectomized female rats, but little information is available regarding the effects of GPER stimulation in male rodents. The aim of the present study was to investigate the effects of the GPER agonist, G1, on consolidation and reconsolidation of inhibitory avoidance (IA) and object recognition (OR) memory in male rats. Animals received vehicle, G1 (15, 75, 150 μg/kg; i.p.), or the GPER antagonist G15 (100 μg/kg; i.p.) immediately after training, or G1 (150 μg/kg; i.p.) 3 or 6 h after training. To investigate reconsolidation, G1 was administered immediately after IA retention Test 1. Results indicated that G1 administered immediately after training at the highest dose enhanced both OR and IA memory consolidation, while GPER blockade immediately after training impaired OR. No effects of GPER stimulation were observed when G1 was given 3 or 6 h after training or after Test 1. The present findings provide evidence that GPER is involved in the early stages of memory consolidation in both neutral and emotional memory tasks in male adult rats.

1. Introduction

Estradiol (17β-estradiol, E_2) induces both rapid (non-genomic) and classical (genomic) actions to alter neuronal structure and function, through activation of multiple signaling pathways and changes in gene expression (Frick & Kim, 2018; Lai, Yu, Zhang, & Chen, 2017; Sheppard, Choleris, & Galea, 2019). For many years, the effects of estrogens on brain function were thought to be mediated by only two types of intracellular estrogen receptors (ERs), ERα and ERβ (Fugger, Foster, Gustafsson, & Rissman, 2000). In the 1990s, the gene for a novel, orphan seven-transmembrane G-protein coupled receptor (GPCR) was cloned (Carmeci, Thompson, Ring, Francke, & Weigel, 1997; Feng & Gregor, 1997; O'Dowd et al., 1998; Owman, Blay, Nilsson, & Lolait, 1996). Initially named G-protein coupled receptor 30 (GPR30), the newly identified receptor was later found by two independent groups to bind E_2, resulting in activation of intracellular signaling (Revankar, Cimino, Sklar, Arterburn, & Prossnitz, 2005; Thomas, Pang, Filardo, & Dong, 2005), then being characterized as an estrogen receptor predominantly located on the cell membrane and designated G protein-coupled estrogen receptor (GPER), which is found in peripheral tissues and cell types including blood vessels, gonadal tissue, and adrenal gland, in addition to the central nervous system (Alexander et al., 2013; Funakoshi, Yanai, Shinoda, Kawano, & Mizukami, 2006).

The abundance and wide distribution of GPER in neuronal

* Corresponding author at: Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite, 500 – 90050-170 Porto Alegre, RS, Brazil.
E-mail address: nadja_s@terra.com.br (N. Schröder).

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membranes in the mammalian central nervous system, including brain areas such as the dorsal hippocampus, make it ideally positioned to regulate learning and memory (Brailoiu et al., 2007; Waters et al., 2015). It is well known that estrogens, particularly E_2, regulate memory formation (Luine & Frankfurt, 2020; Taxier, Gross, & Frick, 2020). E_2 promotes synapse formation as well as neurogenesis in the hippocampus and facilitates memory in female ovariectomized rats and mice (Luine & Frankfurt, 2020; Sheppard et al., 2019). For example, either systemic or intrahippocampal administration of E_2 immediately after training enhance spatial memory of ovariectomized female rats assessed in the Morris water maze. In contrast, E_2 does not affect memory when given 2 h after training, suggesting a specific involvement in the early phase of consolidation (Packard & Teather, 1997a, 1997b). Increasing evidence suggests a similar memory modulating role of E_2 in males, although this has been much less investigated (Taxier et al., 2020). Male rats trained in a spatial water maze task respond similarly to females to intrahippocampal infusions of E_2 (Packard, 1998; Packard, Kohlmaier, & Alexander, 1996). Intracerebroventricular (i.c.v.) administration of the aromatase inhibitor letrozole for 14 days resulted in decreased levels of hippocampal E_2 and impairments in spatial working and object recognition memory both in male and female rats (Marbouti, Zahmatkesh, Riahi, & Sadr, 2020).

Research on the possible role of GEPR in mediating the effects of E_2 has greatly benefited from the development of a selective agonist, G1 (Bologa et al., 2006), and an antagonist, G15 (Dennis et al., 2009). G1 mimics the enhancing effects of E_2 on spine density and increases synaptic transmission in the dorsal hippocampus, in addition to facilitating spatial and object recognition memory in ovariectomized female rodents (Gabor, Lymer, Phan, & Choleris, 2015; Kim, Szinte, Boulware, & Frick, 2016; Kumar & Foster, 2020; Kumar, Bean, Rani, Jackson, & Foster, 2015; Lymer, Robinson, Winters, & Choleris, 2017). G1 also ameliorates deficits in experimental models of memory impairment associated with neurodegeneration in female mice and rats (Kubota, Matsumoto, & Kirino, 2016; Machado et al., 2019).

The role of GPER in regulating brain function in males is much less understood. The density of GEPR in hippocampal synapses is similar in male and female rats, however no evident contribution of GEPR for hippocampal long-term potentiation (LTP) was found (Wang et al., 2018). Training in an object place task activates endogenous GPER in the male rat brain, and this is abolished by G15 administered alone into the perirhinal cortex (Mitchnick et al., 2019). GPER activation by G1 given during a 15-day systemic treatment was able to improve memory for contextual and cued fear conditioning as well as spatial memory assessed in the Morris water maze in middle-aged male mice that show reduced expression of hippocampal GPER. These effects were blocked by co-administration of G15 and were not observed in young adult male mice (Xu, Cao, Zhou, Wang, & Zhu, 2018). G1 also ameliorated spatial memory impairment and reduced neuronal death in a model of traumatic brain injury using male rats (Wang, Pan, Xu, & Li, 2017).

Experiments using exposure of adult male zebra finch hippocampi to an aromatase inhibitor or to G1 and G15 indicate that E2 regulates memory formation and likely increases synaptic strength through GPER activation (Bailey et al., 2017; Bailey, Ma, Soma, & Saldanha, 2013). Thus, aromatase inhibition in the hippocampus impairs spatial memory performance in an ecologically valid food-finding task and decreases levels of hippocampal E2 (Bailey et al., 2013). These effects of E2 may be mediated by GPER activation, involving an increase in PSD95 levels that could influence receptor activity or intracellular signaling pathways to increase synaptic strength (Bailey et al., 2017). Here, we provide the first evidence that acute systemic administration of G1 can enhance memory in young adult male rats though a mechanism dependent on GPER activation.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (290–410 g) were obtained from the institutional breeding facility (CREAL, ICBS, UFRGS, Porto Alegre, Brazil). Animals were housed five per cage in plastic cages with sawdust bedding, and maintained on a 12 h light/dark cycle at a room temperature of 22 ± 1 °C. The rats were allowed ad libitum access to standardized pellet food and water. All experiments took place between 9 AM and 4 PM. All experimental procedures were performed in accordance with the Brazilian Guidelines for the Care and Use of Animals in Research and Teaching (DBCA, published by CONCEA, MCTI) and were approved by the institutional animal care committee (CEUA/UFRGS #36364).

2.2. Drug administrations

Drug solutions were freshly prepared before each experiment. G-1 (1-[4-(6-bromobenzo[1,3]dioxol-5yl)-3a,4,5,9b-tetrahydro-3Hcyclopenta [c]quinolin-8-yl]-ethanone; Cayman Chemical, Michigan, USA), a selective GPER agonist, was dissolved in sunflower seed oil (vehicle) and administered subcutaneously (s.c.) immediately, 3 h or 6 h after the training sessions of memory tasks at the doses of 15, 75 or 150 μg/kg. GPER selective antagonist, G-15 ((3aS,4R,9bR)-4-(6-bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinoline; Cayman Chemical, Michigan, USA) was dissolved in sunflower seed oil and administered s.c. immediately after the training sessions of memory tasks at the dose of 100 μg/kg. Drug doses were chosen based on pilot studies performed in our laboratory using male rats as well as on our previous study performed in female rats (Machado et al., 2019).

Reconsolidation experiment: G-1 dissolved in sunflower seed oil was administered s.c. immediately after inhibitory avoidance (IA) retention test 1 (reactivation session) performed 24 h after training, at the dose of 150 μg/kg.

2.3. Object recognition

Training and testing took place in a 40 cm × 50 cm open field surrounded by 50 cm high walls made of plywood with a frontal glass wall. The floor was covered with sawdust. The objects used for exploration were made of plastic Duplo Lego Toys and had a height of about 10 cm. Objects presented similar textures, colors and sizes, but distinctive shapes. The different objects and their positions were counterbalanced across experiments and behavioral trials, and all objects had a height of about 10 cm. The objects were washed with a 70% ethanol solution between trials. Exploration was defined as sniffing or touching the object with the nose and/or forepaws, sitting on the object was not considered exploration. General training and test procedures followed the methods described in previous reports (Dornelles et al., 2007; Figueiredo et al., 2015; Jobim, Pedroso, Christoff et al., 2012, Jobim, Pedroso, Werenicz et al., 2012). Rats were left to explore the empty arena for 5 min in the first day (habituation). Twenty-four hours after habituation, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 10 cm from the walls. Animals were left to explore the objects during 5 min and the time exploring each object was recorded. On memory retention test trials given 24 h after training, rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. Trials were videotaped and object exploration was measured by an experimenter blind to group treatment assignments, using two stopwatches to record the time spent exploring the objects. A recognition index calculated for each animal was expressed by the ratio $T_N/(T_F + T_N)$ [T_F = time spent exploring the familiar object; T_N = time spent exploring the novel object].

2.4. Inhibitory avoidance

We used the single-trial, step-down IA conditioning as an established model of fear-motivated memory. In IA training, animals learn to associate a location in the training apparatus with an aversive stimulus (footshock). The IA behavioral training and retention test procedures were described in previous reports (Figueiredo et al., 2016; Silva, Garcia, & da Dornelles, 2012). The IA apparatus was a 50 × 25 × 25-cm^3 acrylic box (Albarsch, Porto Alegre, Brazil) whose floor consisted of parallel caliber stainless steel bars (1-mm diameter) spaced 1 cm apart. A 7-cm wide, 2.5-cm high platform was placed on the floor of the box against the left wall. On the training trial, rats were placed on the platform and their latency to step-down on the grid with all four paws was measured with an automatic device. Immediately after stepping down on the grid, rats received a mild footshock (0.4 mA) and were removed from the apparatus immediately afterwards. A retention test trial was carried out 24 h after the training trial. The retention test trial was procedurally identical to training, except that no footshock was presented. Step-down latencies (in seconds) on the retention test trial (maximum 180 s) were used as a measure of IA retention.

In the experiment examining the possible G1 effect on reconsolidation, G1 administration took place immediately after retention test 1 (reactivation session), which was performed 24 h after training. A second test trial (Test 2) was performed 24 h after retention test 1, while a third retention test (Test 3) was performed 24 h after Test 2 (Jobim, Pedroso, Christoff et al., 2012, Jobim, Pedroso, Werenicz et al., 2012).

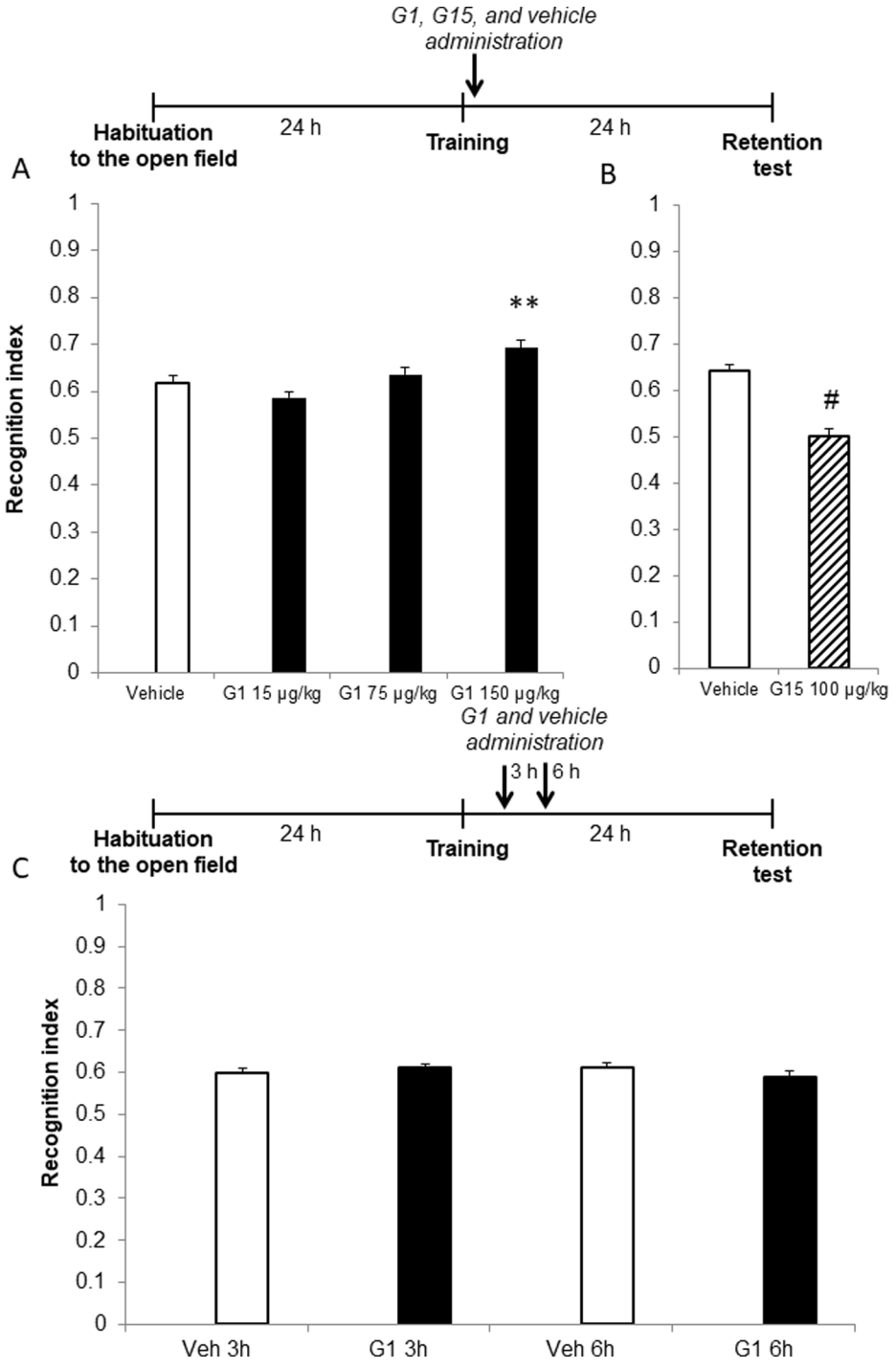

Fig. 1. Effects of G1, a GPER agonist, and G15 a GPER antagonist on object recognition memory. (A) Rats were trained in object recognition task and received vehicle (sunflower seed oil, N = 7), G1 at three different doses (15 µg/kg, N = 8; 75 µg/kg, N = 9; 150 µg/kg, N = 10; s.c.) dissolved in sunflower seed oil, or (B) vehicle (N = 7) or G15 (100 µg/kg, N = 14; s.c.) dissolved in sunflower oil, immediately after training. Retention test was performed 24 h after training. Data are expressed as mean recognition indexes ± S.E. and was analyzed by one-way ANOVA or independent samples *t*-test (G15 vs vehicle immediately after training). (C) Rats were trained in object recognition task and received vehicle or G1 (150 µg/kg, s.c.) dissolved in vehicle, 3 h (Vehicle N = 12; G1 N = 12) or 6 h (Vehicle N = 10; G1 N = 11) after training. Retention test was performed 24 h after training. Data are expressed as mean recognition indexes ± S.E. and was analyzed by independent samples *t*-test comparing groups within each time point (3 h or 6 h). Significant differences between G1or G15 in comparison to Vehicle are indicated as ** $p < 0.01$ and # $p < 0.0001$.

2.5. Statistics

Data from recognition indexes and latencies to step-down are expressed as mean ± standard error of the mean (S.E.). Statistical analyses were performed using SPSS software version 16.0. In the experiments designed to investigate the effects of three different doses of G1 administered immediately after training, comparisons of latencies, recognition indexes, total time exploring objects in the training and test sessions were performed using one-way analysis of variance (ANOVA). Tukey post hoc tests were carried out when necessary. The analyses of the effects of G15 administered immediately after training were performed using independent samples *t*-test. In the experiments designed to examine the effects of delayed G1 administration, comparisons of latencies, recognition indexes, total time exploring objects in the training and test sessions were made using independent samples *t*-test. In the experiment using post-retrieval injections, latencies were analyzed using two-way ANOVA, with drug administration (vehicle or G1) and experimental sessions (test 1, test 2, and test 3) as fixed factors. Tukey post hoc test was carried out to indicate differences between sessions. In all comparisons, $p < 0.05$ was considered to indicate statistical significance.

3. Results

We first examined the effects of different doses of the selective GPER agonist G1 and its antagonist G15, administered immediately after training on object recognition memory consolidation. Statistical comparisons using one-way ANOVA indicated a significant effect when comparing recognition indexes during long-term retention test ($F_{(3, 30)} = 8.18$, $p < 0.0001$, Fig. 1A). Further post hoc comparisons revealed that the animals that received G1 at the highest dose (150 μg/kg) presented a significantly higher, although the increase was small, recognition index than the control group, treated with vehicle ($p = 0.014$), suggesting that GPER stimulation immediately after training discreetly facilitates recognition memory. However, no significant differences were observed when comparing the control group with the groups that received G1 at the doses of 15 ($p = 0.593$) or 75 μg/kg ($p = 0.866$). No significant differences were found when comparing total time exploring objects during the training session ($F_{(3, 30)} = 1.84$, $p = 0.161$; Table 1) or during retention test ($F_{(3, 30)} = 3.08$, $p = 0.052$; Table 1). Independent samples *t*-test revealed that the group treated with the GPER antagonist G15 showed a recognition index significantly lower than the control group in the retention test ($t_{(19)} = 6.23$, $p < 0.0001$, Fig. 1B), suggesting that GPER blockade immediately after training impairs object recognition memory. *T*-test indicated no significant differences in total time exploring the objects during training ($t_{(19)} = -0.662$, $p = 0.519$), or test ($t_{(19)} = 0.418$, $p = 0.687$, Table 1) when comparing G15 and control group (vehicle).

We next decided to investigate the effects of GPER stimulation, 3 or 6 h after training, on recognition memory consolidation, using the dose that proved to affect object memory immediately after training, i.e., 150 μg/kg. Statistical comparisons revealed no significant differences when comparing training recognition indexes (Veh = 0.46 ± 0.017 and G1 = 0.50 ± 0.024; $t_{(22)} = -1.28$, $p = 0.213$) or retention test recognition indexes ($t_{(22)} = -0.71$, $p = 0.484$; Fig. 1C) from animals injected 3 h after training. Comparisons of recognition indexes from animals injected 6 h after training revealed no significant differences in the training session (Veh = 0.48 ± 0.017 and G1 = 0.51 ± 0.018; $t_{(19)} = -1.08$, $p = 0.295$) or in the testing session ($t_{(19)} = 0.88$, $p = 0.389$, Fig. 1 C), suggesting that there is a time-window for GPER participation on recognition memory consolidation. Comparisons of total time exploring both objects during the training session indicated no significant differences between animals injected with vehicle or G1 3 h ($t_{(22)} = -0.14$, $p = 0.886$, Table 2) or 6 h ($t_{(19)} = -1.64$, $p = 0.116$, Table 2) after training. Likewise, comparisons of total time exploring objects during the test revealed no significant differences when groups were injected 3 h ($t_{(22)} = -0.08$, $p = 0.936$, Table 2) or 6 h after training ($t_{(19)} = 0.05$, $p = 0.957$, Table 2).

Our next goal was to determine the effects of GPER stimulation and blockade on inhibitory avoidance. We then administered G1 at different doses and G15 immediately after inhibitory avoidance training. One-way ANOVA indicated a significant difference when comparing latencies to step-down in the long-term retention test ($F_{(3, 36)} = 3.97$, $p = 0.015$; Fig. 2A), but not in the training session (Veh 10.91 ± 2.14, G1 15 μg/kg = 12.83 ± 1.27; G1 75 μg/kg = 11.64 ± 2.12; G1 150 μg/kg = 18.62 ± 2.70; $F_{(3, 36)} = 2.62$, $p = 0.066$). Comparison of latencies in the long-term retention test using post hoc tests indicated that the highest dose of G1 increased latencies to step-down in comparison with the control group ($p = 0.049$; Fig. 2A), while the doses of 15 ($p = 0.933$) and 75 ($p = 1.000$) μg/kg have not affected inhibitory avoidance retention in comparison with controls. Interestingly, differently from the findings on object recognition, GPER antagonist G15 administered immediately after training had no effect on inhibitory avoidance retention, as the latency of the G15-treated group did not differ from that of the control group in the test ($t_{(16)} = -0.006$, $p = 0.995$; Fig. 2B). No significant differences between the groups were observed in the training latencies (Veh 14.38 ± 3.44, G15 100 μg/kg = 17.62 ± 1.83; $t_{(16)} = -0.829$, $p = 0.428$). It should be noted that the differences in basal latency levels in controls observed among experiments are not uncommon and can be often expected in experiments using IA.

We were also interested in evaluating the effects of GPER stimulation, administering G1 at the effective dose of 150 μg/kg, at 3 or 6 h after training on aversive memory consolidation. No significant effects were

Table 1
. Total time exploring both objects in the training and retention test sessions.

Group	Total time exploring objects in the training session (s, mean ± S.E.)	Total time exploring objects in the test session (s, mean ± S.E.)	N
Vehicle	79.74 ± 6.70	73.32 ± 5.92	7
G1 7.5 μg/kg	80.49 ± 7.92	85.47 ± 4.91	8
G1 75 μg/kg	62.32 ± 4.52	78.60 ± 5.12	9
G1 150 μg/kg	71.59 ± 5.74	64.22 ± 5.11	10
Vehicle	66.59 ± 6.05	63.74 ± 10.29	7
G15 100 μg/kg	71.81 ± 5.06	59.14 ± 3.94	14

Rats were trained in object recognition task and received vehicle (sunflower seed oil), G1 at three different doses (15 μg/kg, 75 μg/kg, 150 μg/kg, s.c.) dissolved in sunflower seed oil, or G15 (100 μg/kg, s.c.) dissolved in sunflower oil, immediately after training. Retention test was performed 24 h after training. Data are expressed as mean ± S.E. and was analyzed by one-way ANOVA, followed by Tukey's post hoc test (vehicle vs G1), or independent samples *t*-test (vehicle vs G15). No significant differences were observed.

Table 2
. Total time exploring both objects in the training and retention test sessions.

Group	Total time exploring objects in the training session (s, mean ± S.E.)	Total time exploring objects in the test session (s, mean ± S.E.)	N
Vehicle 3 h	56.05 ± 4.52	50.17 ± 4,70	12
G1 3 h	56.90 ± 3.72	50.65 ± 6.45	12
Vehicle 6 h	60.09 ± 2.56	53.79 ± 5.07	10
G1 6 h	68.74 ± 4.43	53.39 ± 5.20	11

Rats were trained in object recognition task and received vehicle or G1 (150 μg/kg, s.c.) dissolved in vehicle, 3 h or 6 h after training. Retention test was performed 24 h after training. Data are expressed as mean ± S.E. and was analyzed by independent samples *t*-test comparing groups within each time point (3 h or 6 h). No significant differences were found.

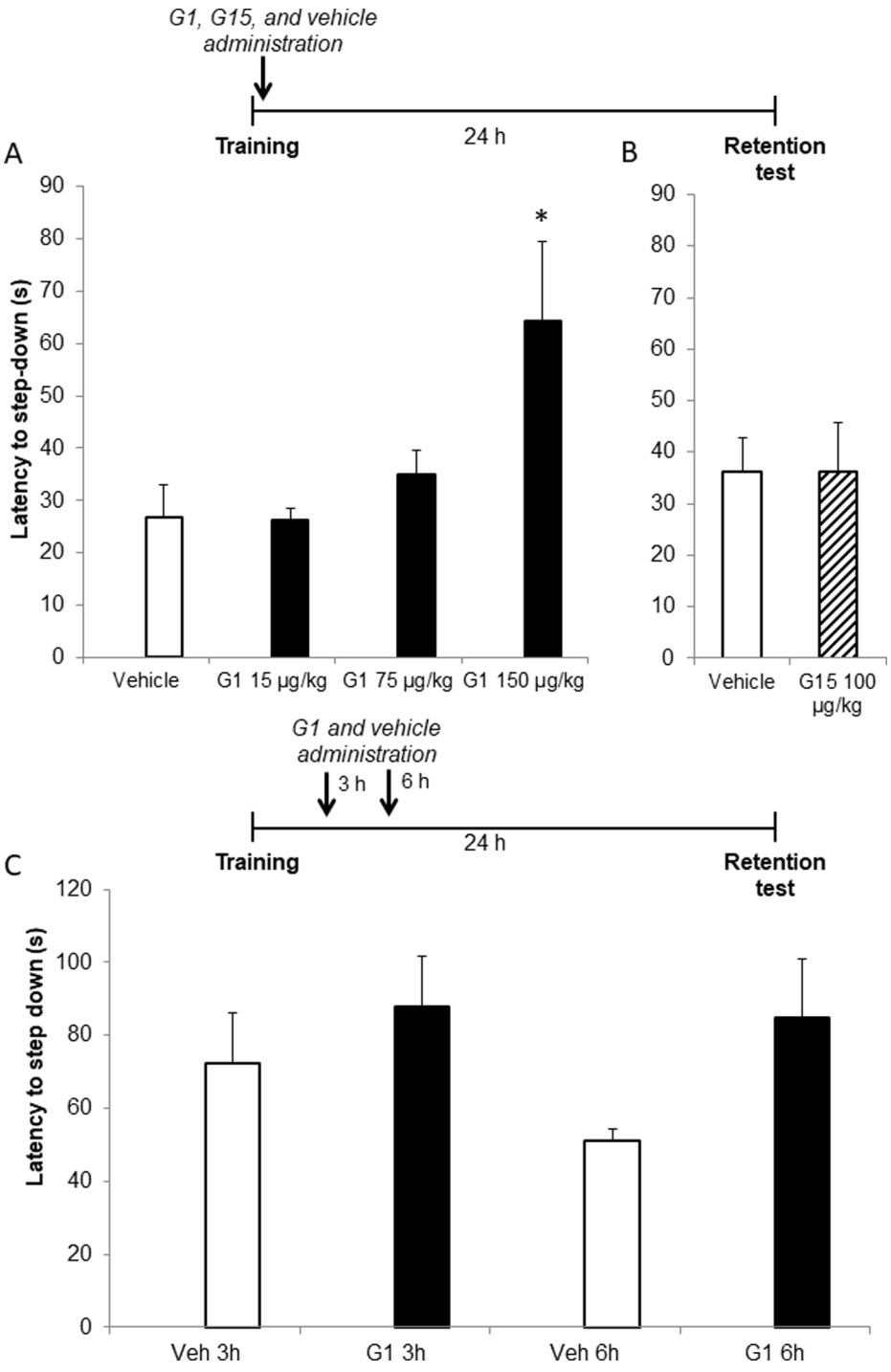

Fig. 2. Effects of G1, a GPER agonist, and G15, a GPER antagonist, on IA memory. (A) Rats were trained in IA and received vehicle (sunflower seed oil, N = 7), G1 at three different doses (15 µg/kg, N = 10; 75 µg/kg, N = 12; 150 µg/kg, N = 11; s.c.) dissolved in sunflower seed oil, or (B) vehicle (N = 7) or G15 (100 µg/kg, N = 11; s.c.) dissolved in sunflower oil, immediately after training. Retention test was performed 24 h after training. Data are expressed as mean latencies to step-down ± S.E. and was analyzed by one-way ANOVA or independent samples *t*-test (G15 vs vehicle immediately after training). (C) Rats were trained in IA and received vehicle or G1 (150 µg/kg, s.c.) dissolved in vehicle, 3 h (Vehicle N = 15; G1 N = 15) or 6 h (Vehicle N = 15; G1 N = 16) after training. Retention test was performed 24 h after training. Data are expressed as mean latencies to step-down ± S.E. and was analyzed by independent samples *t*-test comparing groups within each time point (3 h or 6 h). Significant differences between G1 in comparison to Vehicle are indicated as * $p < 0.05$.

found when latencies to step down from the training session of animals injected 3 h after training ($t_{(28)} = 1.74$, $p = 0.096$; Veh = 13.98 ± 2.13 and G1 = 9.65 ± 1.31) or from the long-term retention test were compared ($t_{(28)} = 0.782$, $p = 0.441$; Fig. 2C). Comparisons between the 6 h-injected groups indicated no significant differences when training latencies were compared ($t_{(29)} = 1.52$, $p = 0.139$; Veh = 16.41 ± 2.61 and G1 = 11.13 ± 2.29), while the comparison between retention test latencies fell short of significance ($t_{(29)} = -2.09$, $p = 0.053$; Fig. 2C).

The next experiment examined the effects of G1 on inhibitory avoidance memory reconsolidation. Two-way ANOVA revealed neither a significant main effect of G1 administration ($F_{(1, 81)} = 1.70$, $p = 0.196$, Fig. 3), or of experimental session (Tests 1–3, $F_{(2, 81)} = 0.44$, $p = 0.646$, Fig. 3). No significant interactions between treatments and sessions were observed (Tests 1–3, $F_{(2, 81)} = 0.027$, $p = 0.974$, Fig. 3).

4. Discussion

In this report, we provide the first evidence indicating that systemic administration of GPER agonist and antagonist in males modulates memory consolidation. Retention of both OR and IA were enhanced by G1, whereas antagonism by G15 selectively impaired OR. Although the size effects in OR experiments were small, they were not negligible under these experimental conditions, and significant statistical differences were found. In contrast, G1 did not affect reconsolidation. The

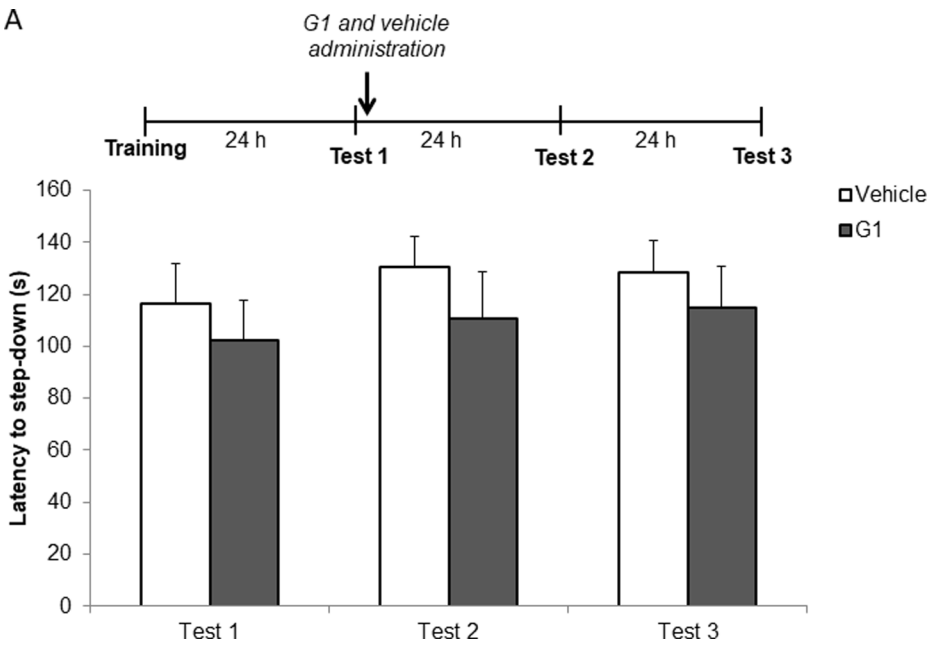

Fig. 3. GPER stimulation does not affect IA memory reconsolidation. Rats were trained in IA and tested for retention 24 h later. Immediately after the 24-h test, vehicle (N = 15) or G1 (150 μg/kg, N = 14) were given s.c. Animals were re-tested for retention 24 h later (Test 2, 48 h after training), and again 72 h after training (Test 3). No significant main effect of drug administration was found. Data are expressed as mean latencies to step-down ± S.E. and was analyzed by two-way ANOVA using drug administration (Vehicle or G1) and experimental session (Test 1, Test 2, and Test 3) as fixed factors.

differential results obtained with G15 in different tasks suggest that emotionally motivated memory traces, such as those produced by IA training, might be less sensitive to modulation by GPER antagonism compared to tasks requiring recognition memory. One can further speculate that the lack of effect of G15 in IA could be related to our choice of dose or might involve possible low levels of endogenous GEPR activation by IA training. In addition, considering the great variability of IA retention test latencies found across experiments in the present study, and that in the experimental set used to test the effects of the GPER antagonist G15, control latencies are in a range of 35 s, the use of training conditions that increase the latencies in the control group could have been more appropriate to reveal a possible impairing effect of G15, as found in the object recognition task.

The selectivity and binding of G1 and G15 to GPER were characterized in detail by the group that developed the compounds, confirming that both compounds are selective and show high affinity at the dose range used here and in other studies (Bologa et al., 2006; Dennis et al., 2009). Although previous studies have not fully characterized the pharmacokinetic dynamics of G1 and G15 in rats or mice, several findings suggest that through peripheral administration, these compounds readily enter the brain to produce central effects. Thus, Dennis et al. (2009) found effects of both G1 and G15 in a mouse model of depression with two successive intraperitoneal (i.p.) injections (G15 followed by G1 or each drug alone followed by E2 or controls). The second injection was given 15 min after the first injection, and behavioral testing was carried out 30 min after the second injection. In a rat model of traumatic brain injury, neuroprotective effects of an i.p. injection of G1 at 50 μg/kg given 30 min after the insult were recently found, and an i.p. injection of G15 at 50 μg/kg blocked the neuroprotective effect induced by E2 (Amirkhosravi et al., 2021). Again in a rat model of traumatic brain injury, intravenous administration of G1 (100 mg/kg) 30 min postinjury led to a reduction of apoptosis and promotion of microglia polarization in the adult rat brain (Pan, Tang, Liu, Feng, & Wan, 2018). Moreover, various central effects of both G1 and G15 on the brain dopaminergic system and MPTP-induced neurotoxicity following s.c. injections were observed in mice (Bourque, Morissette, Côté, Soulet, & Di Paolo, 2012). However, considering that we used only systemic injections of compounds, G1 and G15, and GPER is found in the periphery, we cannot rule out the possibility that behavioral effects may be mediated, at least in part, by peripheral actions.

As well established by James L. McGaugh and coworkers since the 1960s, the use of posttraining drug administration allows for the specific experimental manipulation of memory consolidation, controlling for the possible interference of drug effects on sensorial perception, locomotion, motivation, or other nonspecific aspects of brain function not associated with memory (McGaugh & Izquierdo, 2000; McGaugh, 1966, 2000; Roesler & McGaugh, 2010). Although we did not control for the specificity of the impairing effect of G15 by using delayed infusions, other studies have examined the effects of systemic or intracerebral infusions of G15 on memory and indicated that the effects were not due to drug-related long-term neuronal impairment or lesions. Also, the finding that G1 given at later intervals did not affect inhibitory avoidance retention indicates that G1 effects are time-specific and argues against a role of GPER at later intervals. However, whether G15 given at a later interval could affect memory retention was not determined in our experiments and remains to be verified.

Memory modulation by estrogen signaling shows sexual dymorphism. For example, hippocampal LTP is regulated by ERα in female but not in male rats (Wang et al., 2018). Agonist activation of ERα improves hippocampus-dependent fear memory impairment produced by the antagonist of ER receptors tamoxifen, likely by influencing the early consolidation phase (Lichtenfels et al., 2017). Sex-dependent differences regarding the role of GPER in memory are much less understood. Whereas GPER activation mimicked the postsynaptic effects of E_2 in increasing excitatory postsynaptic responses in the female rat dorsal hippocampus, in males this effect was observed only when an ERβ agonist was used, suggesting a lack of need of GPER in mediating hippocampal actions of E_2 (Oberlander & Woolley, 2016). However, G1 stimulation of GPER during 15 days was able to enhance contextual fear and spatial memory in middle-aged male mice with reduced GPER expression in the hippocampus, and the effect was blocked by G15 (Xu et al., 2018). This same antagonist given acutely into the perirhinal cortex impairs retention of object place memory (Mitchnick et al., 2019). Our results strongly support these few previous studies indicating a role for GPER in influencing memory consolidation in male animals. Importantly, our findings that G1 administration was effective when given shortly after training, but not 3 or 6 h posttraining, places GPER as a system specifically involved in regulating the early phase of memory consolidation in both tasks.

Tamoxifen, which can act either as an antagonist or a partial agonist

of ERα and ERβ estrogen receptors, was shown to impair fear memory reconsolidation when infused into the prelimbic cortex of male rats up to 6 h after reactivation, supporting a role for estrogen signaling in reconsolidation (da Silva, Sohn, Andreatini, & Stern, 2020). However, using a protocol previously shown to reveal crucial mechanisms for IA reconsolidation (Jobim, Pedroso, Christoff et al., 2012, Jobim, Pedroso, Werenicz et al., 2012), we found no effect of G1. It thus remains to be explored by further studies whether GPER plays any role in memory reconsolidation.

In terms of neural systems underlying GEPR regulation of memory, GPR30 is expressed by most of cholinergic neurons in the basal forebrain and acts as an important regulator of basal forebrain cholinergic function, which regulates memory (Hammond & Gibbs, 2011). We hypothesize that the dorsal hippocampus is also importantly involved in memory modulation by GPER, based on previous evidence in female rats (Kim et al., 2016; Kumar & Foster, 2020; Lymer et al., 2017). At the cellular level, the signaling and molecular mechanisms mediating the actions of GPER in memory remain under investigation. Evidence suggests that the effects of G1 depend on c-Jun-N-terminal kinase (JNK), whereas the actions of E_2 are mediated primarily by extracellular signal-regulated kinase (ERK) (Kim et al., 2016). The effects of a 15-day treatment with G1 on long-term depression (LTD) in middle-aged mice involves brain-derived neurotrophic factor (BDNF)/tropomyosin receptor kinase B (TrkB), Akt, and mammalian target of rapamycin (mTor) (Xu et al., 2018).

In summary, we provide novel evidence supporting the view that systemic pharmacological manipulation of GPER modulates consolidation of different types of memory, namely fear-motivated and recognition memories, in male animals. These initial findings could open new avenues of research on the regulation of brain function by estrogens and their G protein-coupled receptors.

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CRediT authorship contribution statement

Lariza Oliveira de Souza: Conceptualization, Methodology, Investigation, Writing – original draft. **Gustavo Dalto Barroso Machado:** Conceptualization, Writing – original draft. **Betânia Souza de Freitas:** Investigation, Validation, Project administration. **Sarah Luize Camargo Rodrigues:** Investigation. **Maria Paula Arakaki Severo:** Investigation. **Patrícia Molz:** Investigation. **José Afonso Corrêa da Silva:** Investigation. **Elke Bromberg:** Conceptualization, Writing – review & editing. **Rafael Roesler:** Conceptualization, Writing – review & editing. **Nadja Schröder:** Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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