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The role of pregnenolone sulphate in spatial orientation-acquisition and retention: An interplay between cognitive potentiation and mood regulation

Fulvio Plescia, Rosa A.M. Marino, Emanuele Cannizzaro, Anna Brancato, Carla Cannizzaro *

Department of Sciences for Health Promotion and Mother and Child Care "Giuseppe D'Alessandro", University of Palermo, V. Vespro 129, 90127 Palermo, Italy

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ABSTRACT

Neurosteroids can alter neuronal excitability interacting with specific neurotransmitter receptors, thus affecting several functions such as cognition and emotionality. In this study, we investigated, in adult male rats, the effects of the acute administration of pregnenolone-sulfate (PREGS) (10 mg/Kg, s. c.) on cognitive processes using the Can test, a non aversive spatial/visual task which allows the assessment of spatial information-acquisition during the baseline training, and of memory retention in the longitudinal study. Furthermore, on the basis of PREGS pharmacological profile, the modulation of depressive-like behaviour was also evaluated in the forced swim test (FST). Our results indicate that acute PREGS induces: an improvement in spatial orientation-acquisition and in reference memory, during the baseline training; a strengthening effect on reference and working memory during the longitudinal study. A decrease in immobility time in the FST has also been recorded. In conclusion, PREGS exerts enhancing properties on acquisition, consolidation and retrieval of spatial information, probably due of improved hippocampal-dependent memory processes. The additional antidepressant effect observed in the FST can provide further evidence in support of the potential of PREGS as a therapeutic tool for the treatment of cognitive deficits associated with mood disorders.

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1. Introduction

A variety of neuroactive steroids may be synthesized in the brain itself, from cholesterol, without the aid of peripheral sources: they have been named "neurosteroids", a variety of pleiotropic molecules that act through both genomic and non genomic mechanisms, broadly affecting several behavioural functions (Baulieu, 1998; Vallée et al., 2001a,b). Among them, pregnenolone and its sulfated ester pregnenolone sulfate (PREGS) have been measured in human plasma and brain tissue (Schumacher et al., 2008) and, despite lacking hormonal action, they still affect neuronal excitability through the modulation of ionotropic receptors. From a pharmacological point of view, PREGS qualified as an "excitatory neuroactive steroid", because it negatively modulates the main inhibitory neurotransmitter receptor in the nervous system, the γ-amimobutyric acid receptor ($GABA_A$), and because it positively regulates the ionotropic glutamate receptor family of ligand gated ion channels such as N-methyl-D-aspartate receptor (NMDA) and amino-3-hydroxy-5methyl-4-isoxazole propionic acid (AMPA) receptors (Akk et al., 2001; Gibbs and Farb, 2004; Gibbs et al., 2006; Wang et al., 2007); it also enhances glutamatergic transmission by acting on presynaptic sigma-1-like receptors (Meyer et al., 2002; Schiess and Partridge, 2005). PREGS plays a critical role in several physiological and paraphysiological processes such as sleep modulation (Darnaudéry et al., 2000; Darbra et al., 2004), emotionality, memory performance, and in age-related neuropsychiatric disorders (Reddy and Kulkarni, 1997, 1998; Reddy et al., 1998; Urani et al., 2001; Phan et al., 2002; Longone et al., 2008). Indeed, decreased concentrations of this neurosteroid have been detected in patients suffering from Alzheimer's disease and multi-infarct dementia, as well as in depression (Näsman et al., 1991; Hillen et al., 2000; Van Broekhoven and Verkes, 2003). These data support the hypothesis that the most prominent, as well as the most proficuous, functions of PREGS deal with the positive modulation of mood and cognition in humans and in animals (Flood et al., 1988,

* Corresponding author at: University of Palermo, Department of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro", Laboratory of Neuropsychopharmacology, Via del Vespro 129, 90127 Palermo, Italy.
E-mail addresses: carla.cannizzaro@unipa.it, carla.cannizzaro@katamail.com (C. Cannizzaro).

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1992, 1995; Vallée et al., 1997, 2001b; Mayo et al., 1993; Reddy, 2011). We usually identify animal cognitive functions as learning and memory: a complex set of neural dynamic processes that mainly consist on acquisition, storage and retrieval of information (Thorpe, 1956; Dudai, 1989). These two differential and complementary functions can be studied in the animal model in order to get information on the neuronal circuitries involved, as well as on the effects of drugs acting in the central nervous system. The formation and storage of long-term declarative memory broadly depend on the interaction between the hippocampus, a region anatomically related to the medial temporal lobe, and the neocortex (Squire and Zola-Morgan, 1991; Eichenbaum, 1991). While in humans the form of memory that strictly depends on the hippocampus is the episodic memory (Amaral et al., 1987), in animals the hippocampus undoubtedly plays a very important role in orientation, in space and in the construction of cognitive maps (Morris et al., 1982; Cain and Saucier, 1996; O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978), engaging the basal forebrain cholinergic system and its target structures (Deiana et al., 2011). Spatial learning and memory can be dissected through application of several maze tasks, including radial arm maze, Y-maze and the water maze, and recently by the Can test. This is a validated, non aversive reward-facilitated task which enables the study of the spatial and visual abilities of the animals, assessing in the same task both working memory, – the temporary storage and manipulation of information – and reference-memory, as a measure of a long-term stable memory traces (Baddeley and Hitch, 1974; Goldman-Rakic, 1996; Fuster, 2001; Dudchenko, 2004; Popoviç et al., 2006). Thus, given these premises, the aim of this research is to analyze the potential effect of PREGS as cognitive enhancer, evaluating its activity during the different phases of learning and memory: acquisition, consolidation and retrieval of spatial information in the Can test. Moreover, on the basis of the pharmacological profile of PREGS, and since several reports show that neurocognitive deficits are comorbid with affective disorders in humans (McIntyre et al., 2013; Godard et al., 2012; Sanchez-Moreno et al., 2009), the modulation of depressive-like behaviour was also evaluated in the forced swim test.

2. Methods

2.1. Animals

Experiments were carried out on adult male Wistar rats (initial weight 200–250, Harlan, Udine, Italy). The animals were housed in a standard plastic cages, two per cage, in a temperature ($22 \pm 2\,^{\circ}C$) – and humidity ($55 \pm 10\%$) – controlled room. Normally, food and water were available ad *libitum*, and the colony was maintained on a 12 h light-dark cycle (8.00–20.00 h).

2.2. Experimental procedures

On the test days, the animals were brought into the laboratory and allowed to acclimatize for at least 60 min prior of the experimental sessions. The experiments were performed in sound-isolated chambers between 8:00 and 14:00. Animal performance was recorded on a videotape placed in an adjacent room. An experimenter, unaware of the different treatments, scored the parameters from the videotape. The devices were thoroughly cleaned before the introduction of each animal to ensure that a particular rat's behaviour was not affected by the detection of another rat scent. All the experiments were carried out in accordance with the current Italian legislation [D.L. 116, 1992] that allows experimentation on laboratory animals only after submission and approval of the research project to the Ministry of Health (Rome, Italy), and in strict accordance with the European Parliament and the Council Directives on the matter (No. 2010/63/EU). All possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

2.3. Pharmacological treatment

Rats were injected with PREGS (Sigma-Aldrich SRL, Milan, Italy) (10 mg/kg; $n = 16$) or vehicle (0.1% Tween 80; $n = 16$) on the day of behavioural testing. PREGS was dissolved in 0.1% Tween 80, and injected (1 ml/kg subcutaneously) two hours before each experimental session, in order to observe a minor inter-individual variability of the effects. Control rats received the same volume (1 ml/kg) of the vehicle at the same time. In this study, we used a single dose of PREGS known to induce the most prominent effects on different behavioural patterns (Yang et al., 2012; Reddy and Kulkarni, 1998).

2.4. Neurocognitive testing

2.4.1. Experimental design

Learning and memory functions were assessed by training the animals in the Can test, a novel motivated, non-aversive spatial-object discrimination task, developed by Popoviç et al. (2001) and Popoviç et al. (2006) and further employed in our previous studies (Cannizzaro et al., 2005; Cannizzaro et al., 2006, 2007). The behavioural protocol consisted of three separate parts: shaping period; spatial orientation-acquisition; spatial orientation-retention. The experiments were performed under 100lux light intensity.

2.4.2. The Can test

The tops of seven soft-drink cans were removed. Cans were painted in white or left in their imprinted colours according to the task administered. The cans were put upside down in a square plexiglas compartment ($100\,cm \times 100\,cm \times 43\,cm$). This allowed their indented bottoms to hold water. The cans were placed on a solid pedestal, which elevated the upper edge of the can to a height of 14 cm. The cans were arranged in a fan shaped pattern, in which the distance from each can to a start point was 70 cm and the distance between the cans was 7 cm. In the task, rats were trained to identify a single rewarded can among a set of seven cans. The reward consisted of 0.3 ml of tap water, using 23 h water deprivation schedule for motivation. When the rat stood on its hind paws and brought its nose up to the level of the top edge of the can, this was considered a "visit". The parameters measured were: (1) "activity", the number of trials on which rats visited at least one can (up to 10 during each experimental session); (2) "Correct responses", the number of trials in which the rat visited the rewarded can first, divided by the activity score (up to 1 per each experimental session); (3) "reference memory errors", the first visits to a non-rewarded can on each trial, divided by the activity score (up to 6 per each experimental session); (4) "working memory errors", repeated visits to the same non-rewarded can on the same trial divided by the activity score. Rats were allowed to drink freely for 20 min at the end of the experimental sessions.

2.4.3. Learning paradigm

2.4.3.1. Shaping period. The shaping period took two days. During this session, the animals were drug-free, and they started habituation by familiarizing with the environment. On the first day, rats were put in the compartment with seven cans. The bottom of each can was filled with 0.3 ml of tap water. The rats were able to explore the compartment and take water from the cans for 20 min. Animals were then removed and placed in their home cages. On the second day, the middle can plus two randomly chosen ones were rewarded with water. The rats had up to 10 min to visit and take water. After

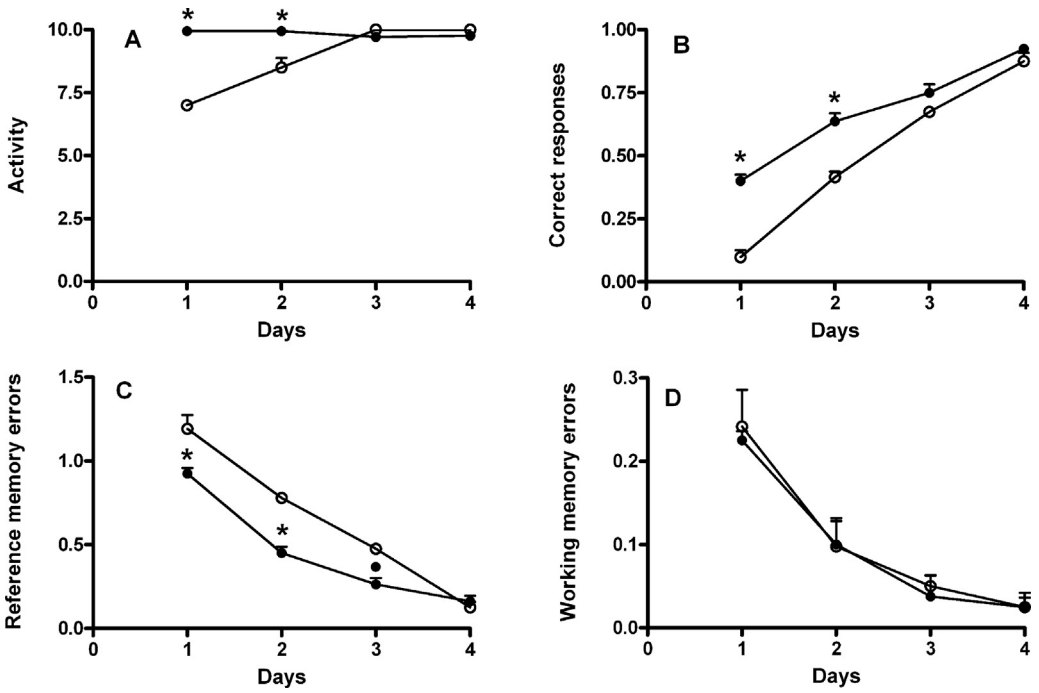

Fig. 1. Can Test: Spatial orientation-acquisition. Effects of PREGS on activity (A), correct responses (B), reference memory errors (C) and working memory errors (D). Each value represents the mean ± SEM of sixteen rats. (◯) Tween, (●) PREGS. *$p < 0.001$, •$p < 0.01$ vs Tween.

a 15 s interval the procedure was repeated again. To avoid possible differences in motivation to perform the Can test, rats that failed to approach and drink from a can over 50% of the trials were excluded.

2.4.3.2. Spatial orientation-acquisition task. 24 hours after the shaping period, two hours following PREGS administration, on four consecutive days (10 trials per day), rats were placed in the compartment where all cans were painted in white and the single rewarded can was always placed in the middle position among the non-rewarded cans. Rats could spend up to 3 min per trial in order to visit and obtain water; once the reward was received the animal was immediately removed from the compartment. During the 15 s interval between trials rats were placed in a small plexiglas box ($50 \times 30 \times 30$).

2.4.3.3. Spatial orientation-retention task. The longitudinal study was performed 14, 28, 42, 56 days after the acquisition task; two hours following PREGS administration, rat performance was tested on a single day. The experimental setting and the task presented were the same as in the acquisition task.

2.5. Forced swim test

Each rat was placed individually in a glass cylinder (40 cm high, 18 cm inside diameter) containing 5–6 l of clean water, depending on the rat size. Water temperature was maintained at 22–23 °C. The animal was forced to swim for 15 min on the 1st day. Animals were then allowed to return to their home cages. On the 2nd day, drugs were administered in the same concentration as in the neurocognitive assessment, 2 h before the swimming session; each rat was then placed into the water and forced to swim for 5 min. The session was videotaped and the duration (in seconds) of immobility and swimming was recorded as measures of depressive-like behaviour. The rat was considered as immobile when it stopped struggling and moved only to remain floating in the water, keeping its head above water.

3. Data analysis

Can Test: a two-way ANOVA was conducted on activity, correct responses, reference memory errors and working memory errors, taken as dependent variables, with "PREGS" (treatment) as a between-subjects factor, and "days" as within-subject factor. When necessary, simple main effects and post hoc comparisons were calculated with Bonferroni post test ($\alpha = 0.05$). Differences were considered statistically significant if $p < 0.05$. Two-tailed Student's t-test for paired-samples was used for comparison of the data between the last acquisition day and the first retention day. Differences were considered statistically significant if $p < 0.05$.

Forced swimming test: evaluation of depressive-like behaviour were conducted performing a two-tailed Student's t-test for unpaired measures on immobility – and swimming time.

4. Results

4.1. Can test

4.1.1. Spatial orientation-acquisition

The effects of PREGS on spatial learning were evaluated at first measuring rats' behavioural strategy during the acquisition phase of the Can Test. The results of a two-way ANOVA for repeated measures including "PREGS-treatment" as the between-subjects factor and "days" as within subjects factor on activity, correct responses, reference memory errors and working memory errors showed a significant effect of time, treatment and their interaction on activity ($F_{(3,90)} = 43.52$, $p < 0.0001$; $F_{(1,30)} = 51.34$, $p < 0.0001$; $F_{(3,90)} = 59.28$, $p < 0.0001$), correct responses ($F_{(3,90)} = 245.65$, $p < 0.0001$; $F_{(1,30)} = 62.44$, $p < 0.0001$; $F_{(3,90)} = 11.50$, $p < 0.0001$), reference memory errors ($F_{(3,90)} = 256.40$, $p < 0.0001$; $F_{(1,30)} = 22.59$, $p < 0.0001$; $F_{(3,90)} = 10.81$, $p < 0.0001$). No significant differences in working memory errors were observed. A post hoc analysis showed that PREGS induced an increase in activity ($t = 13.38$, $p < 0.001$; $t = 6.577$, $p < 0.001$) and in correct responses

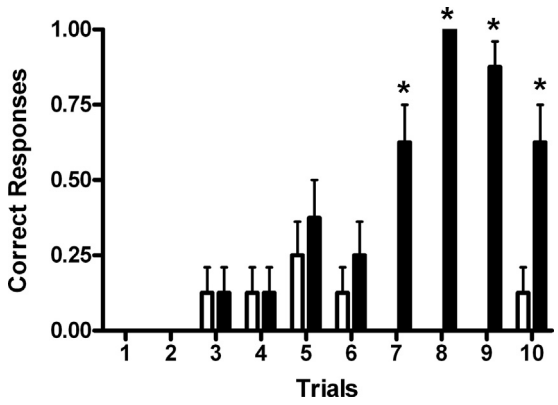

Fig. 2. Can Test: Spatial orientation-acquisition. Effects of PREGS on correct responses trial-by-trial on day 1. Each value represents the mean ± SEM of sixteen rats. (□) Tween, (■)) PREGS. $^{*}p < 0.001$ vs Tween.

($t = 8.173$, $p < 0.001$; $t = 6.027$, $p < 0.001$) on days 1 and 2; and a decrease in reference memory errors on days 1, 2 and 3 ($t = 4.541$, $p < 0.001$; $t = 5.625$, $p < 0.001$; $t = 3.662$, $p < 0.01$) when compared to controls (Fig. 1A, B, C, D). On the last day of the acquisition task, rats from both groups reached the same level of performance. In order to reveal the evolution of performance on day 1 when PREGS effect on the number of correct responses appeared to be more prominent, a two-way ANOVA for repeated measures including "PREGS-treatment" as the between-subjects factor and "trials" as within subjects factor on correct responses was performed. Statistical analysis showed a significant effect of trial, treatment and their interaction on correct responses ($F_{(9,270)} = 10.11$, $p < 0.0001$; $F_{(1,30)} = 85.93$, $p < 0.0001$; $F_{(9,270)} = 12.49$, $p < 0.0001$). A post hoc analysis showed that PREGS induced an increase in correct responses on trials 7, 8, 9 and 10 ($t = 5.637$, $p < 0.001$; $t = 9.020$, $p < 0.001$; $t = 7.892$, $p < 0.001$; $t = 4.510$, $p < 0.001$) (Fig. 2) with respect to controls.

4.1.2. Spatial orientation-retention

The effects of PREGS on memory retention were first evaluated by measuring the differences between the last day of the acquisition and the first day of retention, within the same experimental group. A two-tailed Student's *t*-test for paired samples showed significant differences within the control group on activity, correct responses, reference and working memory errors ($t = 4.227$, $df = 15$, $p < 0.0007$; $t = 4.666$, $df = 15$, $p < 0.0003$; $t = 10.52$, $df = 15$, $p < 0.0001$; $t = 2.179$, $df = 15$, $p < 0.0457$) highlighting, in the first day of the retention task, a loss of the spatial information acquired in the acquisition task. No differences in any parameter examined were recorded in the PREGS group, which showed the same level of performance (Fig. 3A, B, C, D)

Data from the longitudinal study exploring spatial orientation-retention from week 2 to week 8 were analyzed using a two way ANOVA, in order to evaluate the differences between the two experimental groups: a significant effect of time, treatment and their interaction on activity ($F_{(3,90)} = 8.01$, $p < 0.0001$; $F_{(1,30)} = 19.01$, $p < 0.0001$; $F_{(3,90)} = 12.63$, $p < 0.0001$), correct responses ($F_{(3,90)} = 14.28$, $p < 0.0001$; $F_{(1,30)} = 33.72$, $p < 0.0001$; $F_{(3,90)} = 9.26$, $p < 0.0001$), reference memory errors ($F_{(3,90)} = 46.14$, $p < 0.0001$; $F_{(1,30)} = 6.90$, $p = 0.0134$; $F_{(3,90)} = 9.45$, $p < 0.0001$) and working memory errors ($F_{(3,90)} = 8.85$, $p < 0.0001$; $F_{(1,30)} = 28.56$, $p < 0.0001$; $F_{(3,90)} = 8.85$, $p < 0.0001$) were recorded. In details a post hoc analysis demonstrated that, PREGS administration two hours before each test day induced: an increase in activity ($t = 5.436$, $p < 0.001$; $t = 5.426$, $p < 0.001$; $t = 2.861$, $p < 0.001$) and in correct responses ($t = 4.026$, $p < 0.001$; $t = 4.549$, $p < 0.001$; $t = 4.925$, $p < 0.001$) at 14th, 28th and 42nd days after the acquisition phase;

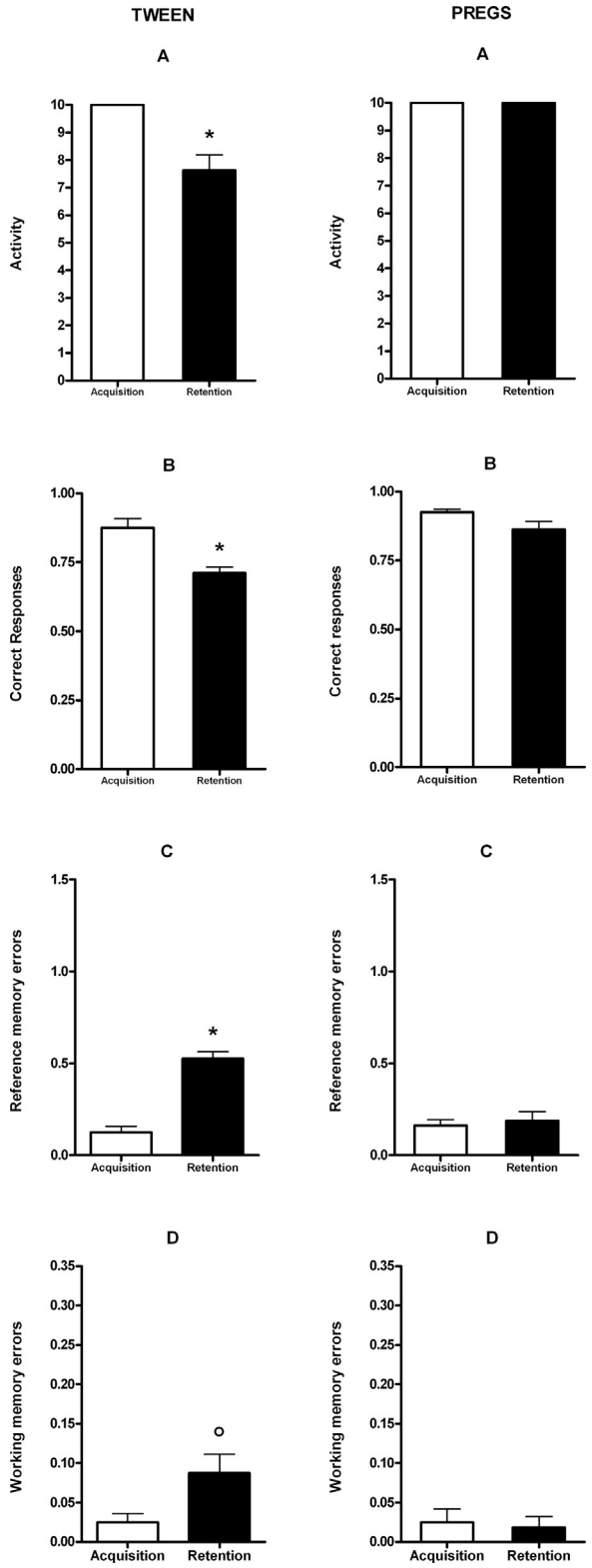

Fig. 3. Can Test: Spatial orientation-retention. Memory retention between the last day of the acquisition and first day of retention on activity (A), correct responses (B), reference (C) and working memory errors (D) on PREGS and control groups. Each value represents the mean ± SEM of sixteen rats. (□) Acquisition, (■) Retention. $^{*}p < 0.001$, $^{\circ}p < 0.05$ vs Acquisition.

a decrease in reference memory errors ($t = 2.627$, $p < 0.05$; $t = 4.487$, $p < 0.001$) and working memory errors ($t = 5.758$, $p < 0.001$; $t = 4.524$, $p < 0.001$) at 14th, 28th days after the acquisition phase, when compared to the control group (Fig. 4A, B, C, D).

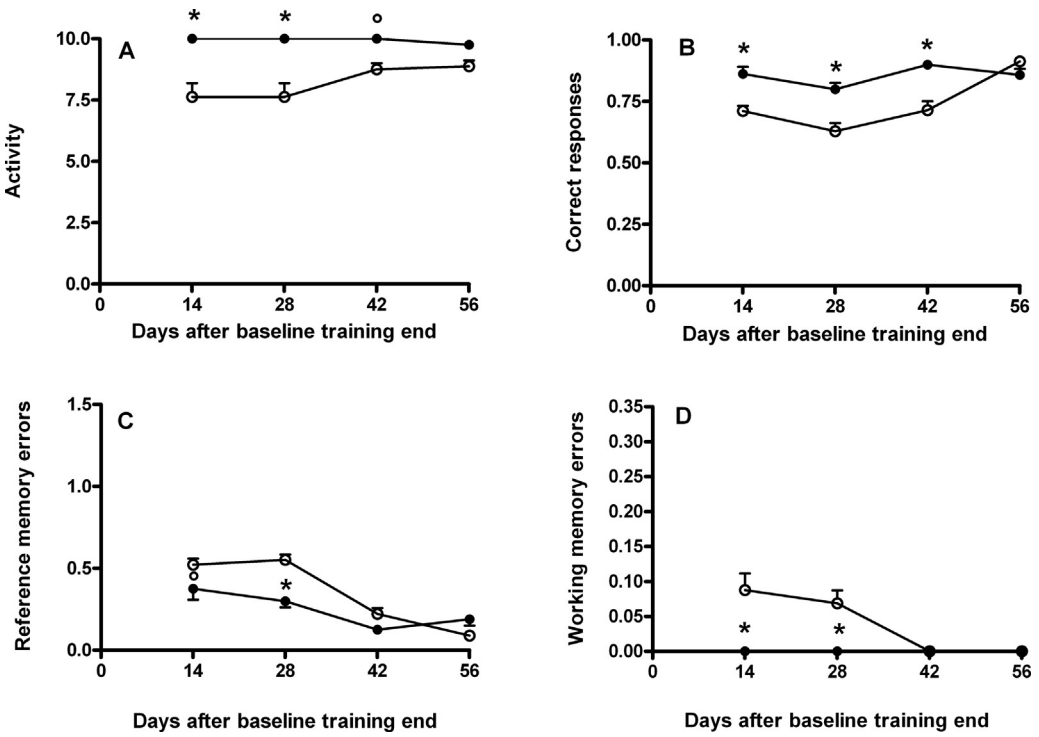

Fig. 4. Can Test: Longitudinal study of spatial orientation-retention at 14, 28, 42, 56 day from spatial orientation-acquisition. Effects of PREGS on activity (A), correct responses (B), reference memory errors (C) and working memory errors (D). Each value represents the mean ± SEM of sixteen rats. (○) Tween, (●) PREGS. *$p < 0.001$, °$p < 0.05$ vs Tween.

4.2. Forced swim test

Rats were tested in the forced swimming test in order to evaluate the effects of PREGS on depressive-like behaviour. The effects of acute PREGS administration on immobility time were analyzed by a two-tailed Student's *t*-test. Our data indicate that PREGS was able to induce a significant reduction in immobility time ($t = 19.85$, $df = 30$, $p < 0.001$), compared to vehicle-treated rats. (Fig. 5).

5. Discussion

In this study, we wanted to investigate, in adult male rats, the consequences of PREGS administration on learning and memory by employing the Can test, a novel non aversive, motivated task, that allows the assessment of both reference and working memory in spatial orientation-acquisition and retention (Popoviç et al., 2001; Popoviç et al., 2006; Cannizzaro et al., 2005; Cannizzaro et al., 2006, 2007). At the same time the consequences of PREGS on rat depressive-like behaviour were tested at the same doses able to affect cognitive processes, by employing the FST, the most popular of all currently available animal models of depression (Page et al., 1999; Urani et al., 2001; Cannizzaro et al., 2008). Our hypothesis is that due to its pharmacological profile, PREGS can potentiate discrete cognitive functions and at the same time produce an enhancement in the emotional processing.ç

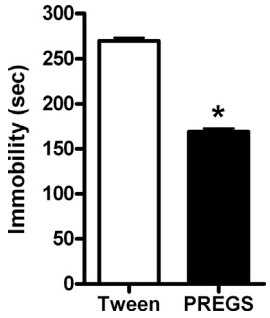

Fig. 5. Forced Swim Test: Effects of PREGS treatment on immobility and swimming time. Each value represents the mean ± SEM of sixteen rats. (□) Tween, (■) PREGS. *$p < 0.001$ vs Tween.

The first part of the research is aimed at assessing PREGS effects on spatial orientation-acquisition. Indeed, the ability to learn and remember spatial locations, and to associate them with other stimuli, is essential for adaptive behaviour in many species. Whereas some spatial tasks can be solved purely on the basis of egocentric information that will change every time the animal moves, other spatial tasks require encoding the relationship between salient features of the environment to create an allocentric representation that is independent on the animal's current location: such a representation has been termed a "cognitive map" (O'Keefe and Nadel, 1978). And the construction of a cognitive map is just requested in the first paradigm of the Can test: in this experiment rats have to search for the rewarded can just on the basis of its central position, and spatial discrimination is correlated to the activation of the hippocampus, a crucial area for map-like or relational memory formation (Bingman et al., 1998; Burgess et al., 1999; Eichenbaum et al., 1994; Eichenbaum et al., 1990; O'Keefe and Nadel, 1978). Here, PREGS administration induced an increase in the number of correct responses, and a decrease in the number of reference memory errors thus improving processing of spatial information in a new environment, and the consequent acquisition of a spatial cognitive map. PREGS effect is more prominent in the first day of the acquisition task, when correct response measure was well above chance. It was thus informative to examine trial-by-trial performance in the two experimental groups. Indeed, PREGS-treated rats showed a different pattern of learning depending on the evolution of their performance along the trials displaying a significant increase in the rate of correct responses in the last five trials. This result puts even more in evidence the facilitatory effect

of PREGS that make the animals more prompt to record and integrate the spatial information correlated to their navigation in the arena, in order to give the adaptive response.

When the animals were analyzed longitudinally in the spatial orientation-retention phase, the consolidation and retrieval of former spatial information were requested at two week-time from the former acquisition task, every two weeks for four times. Again, interestingly, PREGS induced an increase in the number of correct responses and a decrease both in the number of reference- and in the number of working- memory errors, compared to controls. Moreover, comparisons within the same group between the last day of spatial-acquisition and the first day of retention showed that PREGS-treated group was able to retain the high level of performance as shown by the same degree of correct responses and reference- and working- memory errors to two weeks apart. The controls, on the contrary, at the first session of the longitudinal evaluation, displayed a lower number of correct responses, and higher number of reference- and working memory errors with respect to the acquisition phase. These results indicate that PREGS interferes positively not only with the acquisition of spatial information helpful for the following formation of the cognitive map, but also with the temporary storage of the information in brain areas involved in the encoding and consolidation phases that permit the long-term storage and retrieval of spatial memory traces. Numerous reports exist on the memory enhancing properties of sulphated neurosteroids, and several studies indicate a direct effect of PREGS on NMDA and AMPA receptors within the hippocampus which would result in enhancement of long-term potentiation (LTP) in the pyramidal neurons (Mathis et al., 1996; Akwa et al., 2001). On the other hand, PREGS also acts as a negative allosteric modulator of GABA-A receptor. This makes it able to induce a disinhibition of the cholinergic neurons of the nucleus basalis magnocellularis that project to the hippocampus and cerebral cortex, thus strengthening the molecular substrates that underly cognition (Darnaudéry et al., 1998; Pallarés et al., 1998; Vallée et al., 2001a; Mayo et al., 2003).

Interestingly, in spatial discrimination-acquisition, PREGS affected deeply reference memory whilst it influenced working memory only during the longitudinal study, and in particular in the first two longitudinal experimental sessions. It is well known that working memory is to be distinguished as a separate process from reference memory: whilst working memory requires the ability to maintain trial-specific information for a limited period of time, so that environmental stimuli can be responded to in a flexible manner, reference memory requires the ability to learn the correct fixed response to a stimulus (Sanderson and Bannerman, 2012). PREGS-induced reduction in the number of working memory errors in the longitudinal evaluation of spatial orientation-retention, which was not observed in the acquisition phase, allows us to speculate on the highlighting of two dissociable forms of spatial information processing, both of which depend on the hippocampal activation but need different molecular components for their expression: a rapid form of information processing which underlies, or at least contributes to, spatial working memory performance that is known to be dependent on GluA1-containing AMPA receptor recruitment (Sanderson et al., 2007, 2009); and a gradually acquired, or incrementally strengthened, spatial reference memory mechanism that is NMDA-dependent and GluA1-containing AMPA receptor independent (Collingridge et al., 1983). LTP as a matter of fact is a NMDA-dependent process, but its maintenance, at least in part, depends on the traslocation of additional AMPA receptors into the postsynaptic membrane (Kessels and Malinow, 2009; Malinow and Malenka, 2002).

Our hypothesis is that, during the learning phase, PREGS is able to induce LTP through its action on NMDA receptors, strengthening the acquisition of spatial information and the setting up of long-term memory in order to adapt a strategy towards a new task in a new environment. At the induction of LTP, intracellular specific protein activation occurs with the following induction of AMPA receptor recruitment to the synapse (Shi et al., 1999; Hayashi et al., 2000; Lu et al., 2001). Thus, in the longitudinal study, PREGS could promote the activation of AMPA receptors that appear to contribute more to a form of short-term potentiation useful to the strengthening of spatial working memory (Erickson et al., 2010). However, given this possible explanation of the behavioural data, further investigation is needed to deeply explore the intrinsic mechanisms underlying the observed effects.

It has repeatedly been hypothesized that cognitive processes and anxiety- and depressive-related behaviour may interact in a fundamental manner. The literature though is extremely confusing. Some authors suggest that a cognitive dysfunction represents the primary revealing feature of pathological anxiety and depression (Ferreri et al., 2011; Ohl, 2005). On the other hand, studies on animals using pharmacological and genetic tools have shown that stress facilitates, and might even be indispensable for, good learning and memory performance (Oitzl and de Kloet, 1992; Oitzl et al., 2001; de Kloet et al., 2002). Thus, given these controversial findings, it was of great interest to us investigating whether PREGS, at the same dosage able to affect cognitive processes induced a modification in the affective behaviour in rats.

To do that, we employed the forced swim test, that is the most widely used and one of the most reliable and consistent animal test predictive of drug antidepressant activity in rodents (Cryan et al., 2005; Petit-Demouliere et al., 2005). Our results show that PREGS was able to reduce immobility time and increase swimming, in the FST exerting an antidepressant activity. These results appear to be consistent with the antidepressant effects described for PREGS in other studies in animals and humans (Urani et al., 2001; Wolkowitz et al., 1999; Reddy et al., 1998). Besides its activity as an allosteric modulator of NMDA receptor subtype and of GABA receptor/chloride ionophore, PREGS activity in the hippocampus might be dependent on presynaptic metabotropic sigma-1-like receptors. And, in this context, there is little doubt that the activation of the sigma-1 receptors and the following interaction with noradrenaline and serotonin transmission could explain the antidepressant effect exerted by PREGS (Maurice et al., 1996; Delgado and Moreno, 2000).

In conclusion, the enhancement of neurosteroids in the brain seems to be necessary to maintain normal excitatory synaptic transmission and plasticity in cerebral areas involved in the modulation of affective and cognitive behaviour. On the other hand, cognition, as defined by attention, memory and executive function, is impaired in depression. Hence, although further studies are required to better define the pharmacological profile of PREGS, on the basis of the present behavioural data, we suggest that PREGS possesses potential therapeutic activity in memory dysfunctions associated with depressive symptoms in humans.

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