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Attenuation by a sigma$_1$ (σ_1) receptor agonist of the learning and memory deficits induced by a prenatal restraint stress in juvenile rats

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1 Stress during pregnancy results in complex neurochemical and behavioral alterations throughout the offspring lifetime. We here examined the impact of prenatal stress (PS) on memory functions in male and female offspring and report the efficacy of a selective sigma$_1$ (σ_1) receptor agonist, igmesine, in alleviating the observed deficits.

2 Dams received an unpredictable 90-min duration restraint stress from gestational day E17 to E20. Learning was examined in offspring between day P24 and P36 using spontaneous alternation in the Y-maze, delayed alternation in the T-maze, water-maze learning and passive avoidance.

3 Both male and female PS rats showed impairments of spontaneous and delayed alternation performances. Acquisition of a fixed platform position in the water-maze was unchanged in PS rats, but the probe test revealed a diminution of time spent in the training quadrant. Acquisition of a daily changing platform position demonstrated impaired working memory for male and female PS rats. Finally, passive avoidance deficits were observed.

4 Pretreatment with the selective σ_1 agonist igmesine (1–10 mg kg^{-1} i.p.) reversed the PS-induced learning deficits in offspring rats for each test. The σ_1 antagonist BD1063 failed to affect performances alone but blocked the igmesine effect, confirming the involvement of the σ_1 receptor.

5 PS thus induces delayed memory deficits, affecting spatial and nonspatial, short- and long-term memories in juvenile male and female offspring rats. Activation of the σ_1 neuromodulatory receptor allows a significant recovery of the memory functions in PS rats.
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Keywords: Prenatal stress; σ_1 receptor; learning and memory; spontaneous alternation; delayed alternation; water-maze learning; passive avoidance

Abbreviations: ACTH, adrenocorticotropin releasing hormone; AL, adjacent left quadrant; ANOVA, analysis of variance; AR, adjacent right quadrant; CRF, corticotropin-releasing factor; HPA, hypothalamo-pituitary-adrenal; ITI, intertrial time interval; NMDA, *N*-methyl-D-aspartate; O, opposite quadrant; PS, prenatal stress; SAM, senescence-accelerated mouse; T, training quadrant

Introduction

Increasing evidence from animal studies shows that prenatal exposure to stressful events directly affects the neurophysiological development of the fetus with deleterious consequences observable throughout lifetime (Weinstock, 1997; 2001; Kofman, 2002). A prenatal stress (PS) results in the hyperactivation of the maternal hypothalamo-pituitary-adrenal (HPA) axis, leading to enhanced production of stress hormones. Consequently, PS offspring exhibits elevated neuroendocrine responses to stress, including elevated secretions of corticotropin-releasing factor (CRF), adrenocorticotropin-releasing hormone (ACTH) and corticosterone (Peters, 1982; Maccari *et al*., 1995; Weinstock *et al*., 1998), and a reduction in the density of corticosteroid receptors in the hippocampus (Weinstock, 1997; Vallée *et al*., 1999). Finally, the hippocampus appears as a brain structure sensitive to PS (Weinstock, 1997; 2001; Vallée *et al*., 1999) and PS offspring shows from infancy to senescence and throughout adulthood marked memory deficits in numerous, and particularly hippocampal-dependent, behavioral tasks (Szuran *et al*., 1994; Lordi *et al*., 1997; Vallée *et al*., 1999). We previously reported that a maternal restraint stress, administered during the last week of gestation, results in marked learning and memory impairments in 4-week-old offspring rats (Gué *et al*., 2004). Indeed, juvenile male and female PS rats shows impairments of spatial working memory, assessed using the spontaneous alternation behavior in the Y-maze; short-term memory, assessed using a delayed alternation test in the T-maze; and contextual long-term memory, assessed using a passive avoidance procedure (Gué *et al*., 2004).

Alleviating PS-induced cognitive deficits will require a strategy allowing both a pharmacological and developmental intervention, restoring rapidly the impaired learning abilities and normalizing the neurophysiologic and neuroendocrine deficits observed in the PS offspring. Among the candidate systems, the sigma$_1$ (σ_1) receptor may be a promising target.

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The σ_1 receptor is an intracellular neuronal protein associated with endoplasmic reticular, nuclear, mitochondrial, and plasma membranes (Alonso *et al*., 2000; Hayashi *et al*., 2000). In basal conditions, the σ_1 receptor forms a heterotrimeric macromolecular complex with the inositol trisphosphate receptor and an ankyrin B cytoskeleton anchor protein (Hayashi *et al*., 2000; Hayashi & Su, 2001). Selective σ_1 receptor ligands potently modulate intracellular Ca^{2+} mobilization by translocation of the σ_1 receptor/ankyrin complex towards organelles or plasma membranes. In the vicinity of the latter, the σ_1 receptor activation results in a modulation of extracellular Ca^{2+} influx (Hayashi *et al*., 2000; Hayashi & Su, 2001). Through such a mechanism or, putatively, *via* direct interactions with the membrane receptors, activation of the σ_1 receptor also results in modulation of several responses to neurotransmitters (Monnet *et al*., 1992; Gonzalez-Alvear & Werling, 1994). The σ_1 system has recently been proposed by Su & Hayashi (2003) to constitute a 'unique intracellular amplifier system for signal transduction'. This nonselective, but efficient, neuromodulation affects learning and memory processes, response to stress or depression (for reviews, Maurice *et al*., 1999b; 2001). In particular, σ_1 receptor agonists efficiently alleviate not only pharmacological models of amnesia, induced in rodents by systemic administration of glutamatergic or cholinergic antagonists for instance, but also pathological models of amnesia. Aged rats or mice, senescence-accelerated (SAM) mice, or rodents injected centrally with β-amyloid peptides, develop learning and memory impairments that can be reversed by σ_1 receptor agonists (Maurice *et al*., 1996; Maurice, 2001; Tottori *et al*., 2002; Phan *et al*., 2003).

In the present study, the pharmacological effect of the selective σ_1 receptor agonist igmesine (Roman *et al*., 1990) was tested against the learning and memory deficits observed in juvenile PS rats. Pregnant dams were submitted to a daily restraint stress between E17 and E20. Male and female PS offspring rats were examined from P24, for their learning ability in: the spontaneous alternation test in the Y-maze, the delayed alternation test in the T-maze, the place learning test in the water-maze, following a procedure that examines the reference and working memory components separately, and a passive avoidance procedure. Igmesine (1–10 mg kg^{-1} i.p.) was administered before each behavioral session. The involvement of the σ_1 receptor was checked using the preadministration of the selective σ_1 receptor antagonist BD1063 (de Costa *et al*., 1992).

Methods

Animals and PS procedure

Adult virgin Sprague–Dawley female rats (Depré, Doulchard, France) weighing 240 g were group-housed (10 per cage) during 10 days, to coordinate their estrous cycle and then individually housed for a whole estrous cycle (4 days), in the presence of a sexually experienced male rat, weighing 400 g. The presence of a vaginal plug was considered as embryonic day E0. Female rats were received at our animal facility at E13. They were assigned randomly to PS or control groups, individually housed in plastic breeding cages, allowed *ad libitum* access to food and water, and maintained on a constant 12 h : 12 h light : dark cycle (lights on at 0800), at constant room temperature (21°C) and humidity (50%).

The PS procedure was carried out as previously described (Gué *et al*., 2004). Between E17 and E20, dams received a semirandomized restraint stress procedure. Repeatedly restrained animals were placed in Plexiglas transparent cylinders (20 cm length, 7 cm diameter) under bright light during 90 min per day, for 4 consecutive days. To make the stressor as unpredictable as possible, the 90-min restraint period was administered either as a single 90 min block, as two 45 min blocks with a delay of 4 h, as a 60 and 30 min blocks with a delay of 4 h, or as three 30 min blocks with delays of 4 and 1 h between each block, occurring at various times during the day. Control dams were also handled but never placed in restraint tubes. Dams were allowed to deliver naturally on E21, which was considered as P0. After birth, all litters were culled to 10 pups, with five males and five females whenever possible. Their biological mothers raised the pups. As previously observed (Gué *et al*., 2004), the two groups were found no different on measures of weight gain, litter size at birth, offspring mortality or general behavioral outcome. Body weights were measured at P1 and regularly during experimental tests. Litters were weaned at P21. They were separated from the mothers, sexed, weighed and housed six to a cage ($52 \times 35 \times 18\,cm^3$) in same-sex group but originating from different litters, in order to avoid a possible litter-related effect.

Between P24 and P36, all juvenile animals went through the experimental sessions. At P24, animals were submitted to the spontaneous alternation test in the Y-maze. At P25 or P26, they were submitted to the delayed alternation test in the T-maze. From P27 to P31, rats were submitted to place learning in the water-maze, with a probe test 1 h after the last swim session on P31. From P32 to P34, animals performed the working memory version of the water-maze. Finally, they were examined for a step-through type passive avoidance response at P35-P36. Animals were trained at P35 and the retention session was carried out after 24 h. Experiments were carried out between 0900 and 1700, in a sound-attenuated and air-regulated experimental room, to which rats were habituated at least 30 min before each experiment. All animal procedures were conducted in strict adherence to the European Communities Council Directive of 24 November 1986 (86-609/EEC).

Drugs

(+)-cinnamyl-1-phenyl-1-*N*-methyl-*N*-cyclopropylene hydrochloride (igmesine) was provided by Dr François J. Roman (Pfizer GRD, Fresnes, France) and *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*,*N*′,*N*′-trimethylethylenediamine (BD1063) by Dr Wayne D. Bowen (NIDDK, NIH, Bethesda, MD, USA). The σ_1 ligands were solubilized in distilled water and injected intraperitoneally (i.p.) in a volume of 100 μl per 100 g of body weight. Pharmacological treatment is shown for each following test procedure.

Spontaneous alternation performance in the Y-maze

Recording the spontaneous alternation behavior in a Y-maze assessed spatial working memory performance (Maurice *et al*., 1994; 1996; 1998). The maze was made of black painted wood. Each arm was 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converged at an equal angle. Each

rat was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries, including possible returns into the same arm, was recorded using an Apple IIe computer. An alternation was defined as entries into all three arms on consecutive occasions. The number of maximum alternations was therefore the total number of arm entries minus two and the percentage of alternation was calculated as (actual alternations/maximum alternations) $\times 100$. In addition, the total number of arms entered during the session was also determined. The σ_1 ligands, igmesine and/or BD1063, were administered i.p. 20 min before the 8-min session.

Delayed alternation performance in the T-maze

The maze was made of black polyvinylchloride. Two short arms (20 cm long) extended from a longer alley (40 cm long). Arms were 10 cm wide and enclosed with 25 cm high walls. The test consisted in two trials separated by an intertrial time interval (ITI) of 1 h. During the first, acquisition trial, one short arm was closed. Rats were placed at the end of the long alley and allowed to visit the maze for 10 min. During the intertrial time, rats were housed in their home cage. During the second, retention trial, animals were placed again in the maze for 2 min, having free access to all three arms. The number of visit and time spent in each arm were measured. Results were expressed as ratio of the time spent in the novel (initially closed) arm over the time spent in the previous arm and as ratio of the number of entries into the novel over previous arm. The σ_1 ligands were administered i.p. 20 min before the second, 2-min duration, session.

Place learning in the water-maze

The maze was a circular pool (160 cm diameter, 40 cm height) that the videotracking systems could arbitrarily divide into four quadrants. The water temperature ($24 \pm 2^{\circ}$C), light intensity, external cues in the room, and water opacity, obtained by suspension of lime carbonate, were rigorously reproduced. A transparent Plexiglas platform, 10 cm in diameter, could be immersed 2 cm under the water surface at the center of each quadrant during training sessions. This quadrant was termed the training (T) quadrant and the others opposite (O), adjacent right (AR), and adjacent left (AL) quadrants, during the subsequent retention session. Swimming was recorded using a CCD camera connected to a computer, trajectories being analyzed in terms of latencies and distances using the Videotrack® (version NT) software (Viewpoint, Champagne-au-Mont-d'Or, France).

The behavioral procedure was carried out as follows. Animals were trained to learn a fixed location of the invisible platform during 5 days. They were then submitted to a probe test with the platform being removed from the pool. Then, animals were submitted to a working memory procedure with platform location changing every day, during 3 days. Training consisted of three swims per day from days 1 to 5, with an intertrial time interval of 10 min. Start positions, set at each limit between quadrants, were randomly selected for each animal. Each animal was allowed a 90 s swim to find the platform and was left for a further 20 s on the platform. Animals failing to find the platform were placed on it manually. On the fifth day (P32), 1 h after the last swim, the platform was removed and each animal was allowed a free 60 s swim. The percentage of time spent in each quadrant was determined. The working memory test was carried out, as previously described (Yamada *et al*., 1999) during 3 days from days 6 to 8 and consisted in four trials per day with 2-min ITI. The platform location changed every day but not among trials. Start positions, set at each limit between quadrants, were randomly selected for each animal. Each animal was allowed a 90 s swim to find the platform and was left for a further 20 s on the platform. Data represent the mean performance over days for each trial. Each trial is an informative sample trial in which the animal is allowed to swim to the platform in its new location. Rats show a rapid involvement of the working memory between the first and second trial, which allow measuring the quality of spatial working memory (Yamada *et al*., 1999). The σ_1 ligands were administered 20 min before the first swim trial on each training day.

Step-through passive avoidance test

The apparatus consisted of an illuminated compartment with white polyvinylchloride walls ($15 \times 20 \times 15\,cm^3$ high), a darkened compartment with black polyvinylchloride walls ($15 \times 20 \times 15\,cm^3$ high) and a grid floor. A guillotine door separated each compartment. A 60 W lamp positioned 40 cm above the apparatus lit the white compartment during the experimental period. Scrambled foot shocks (0.3 mA for 3 s) were delivered to the grid floor using a shock generator scrambler (Lafayette Instruments, Lafayette, MA, U.S.A.). The guillotine door was initially closed during the training session. Each rat was placed into the white compartment and after 5 s, the door was raised. When the rat entered the black compartment and placed all its paws on the grid floor, the door was gently closed and the scrambled foot shock was delivered for 3 s. The step-through latency and the number of vocalizations were recorded. The number of vocalizations did not differ among groups, indicating that shock sensitivity was identical. The retention test was carried out 24 h after training. Each rat was placed again into the white compartment and after 5 s, the door was raised. The step-through latency was recorded up to 300 s. The σ_1 ligands were administered 20 min before the training session, injections not being repeated before the retention session.

Statistical analyses

All behavioral data were expressed as mean $\pm$ s.e.m., except for passive avoidance results. Step-through latencies did not show a normal distribution, since a cutoff time was set. They were thus represented as median and interquartile range. The median swim latencies during the repeated acquisition procedure (days 1–5 of the water-maze test) was calculated per day and then expressed as mean $\pm$ s.e.m. for each experimental group. Data for male and female offspring were analyzed separately using the Dunnett's multiple comparisons test after a one-way analysis of variance (ANOVA, F values). Latencies were analyzed over trials using the nonparametric Friedman repeated measures test (Fr values), comparisons between groups being made using Dunn's test. Step-through latencies were analyzed using the Kruskal–Wallis non-parametric ANOVA (KW values), group comparisons being made

with Dunn's nonparametric multiple comparisons test. The level of statistical significance was $P<0.05$.

Results

Spontaneous alternation performances

On day P24, analyses of spontaneous alternation performances revealed significant effects for both males ($F(9,83)=41.67$, $P<0.0001$; Figure 1a) and females ($F(6,63)=2.37$, $P<0.05$; Figure 1b). PS resulted in a highly significant decrease of alternation percentage (black columns, $P<0.001$, Dunn's test), which diminished to 56%, that is, close to the random choice, suggesting that PS rats failed to develop a spatial strategy to explore the maze. The pretreatment with igmesine resulted in a dose-dependent reversion of the alternation deficits, with no effect in non-PS rats. Indeed, PS offspring treated with the highest doses of igmesine (3, 10 mg kg^{-1} for male and 10 mg kg^{-1} for female) showed alternation percentage returning to control values (Figure 1a and b). The involvement of the σ_1 receptor was checked in male offspring rats using a pretreatment with BD1063. The σ_1 receptor antagonist failed to affect the performance of non-PS or PS offspring male rats, but highly significantly blocked the igmesine effect (Figure 1a). The exploratory activity was examined through the total number of arms entered during the session (Figure 1c and d). A significant effect was measured for males ($F(9,83)=18.29$, $P<0.0001$; Figure 1c) and females ($F(6,63)=3.87$, $P<0.05$; Figure 1d). For both male and female offspring, it appeared that the highest dose of igmesine (10 mg kg^{-1}) decreased the number of arms entered, mainly in non-PS rats.

Delayed alternation behavior

On day P25, offspring rats were submitted to the delayed alternation test, with an ITI of 1 h. Analysis of the ratio of time spent in the novel vs previous arm revealed a significant effect for both male ($F(9,79)=4.31$, $P<0.001$; Figure 2a) and female ($F(6,57)=2.54$, $P<0.05$; Figure 2b) rats. Indeed, control

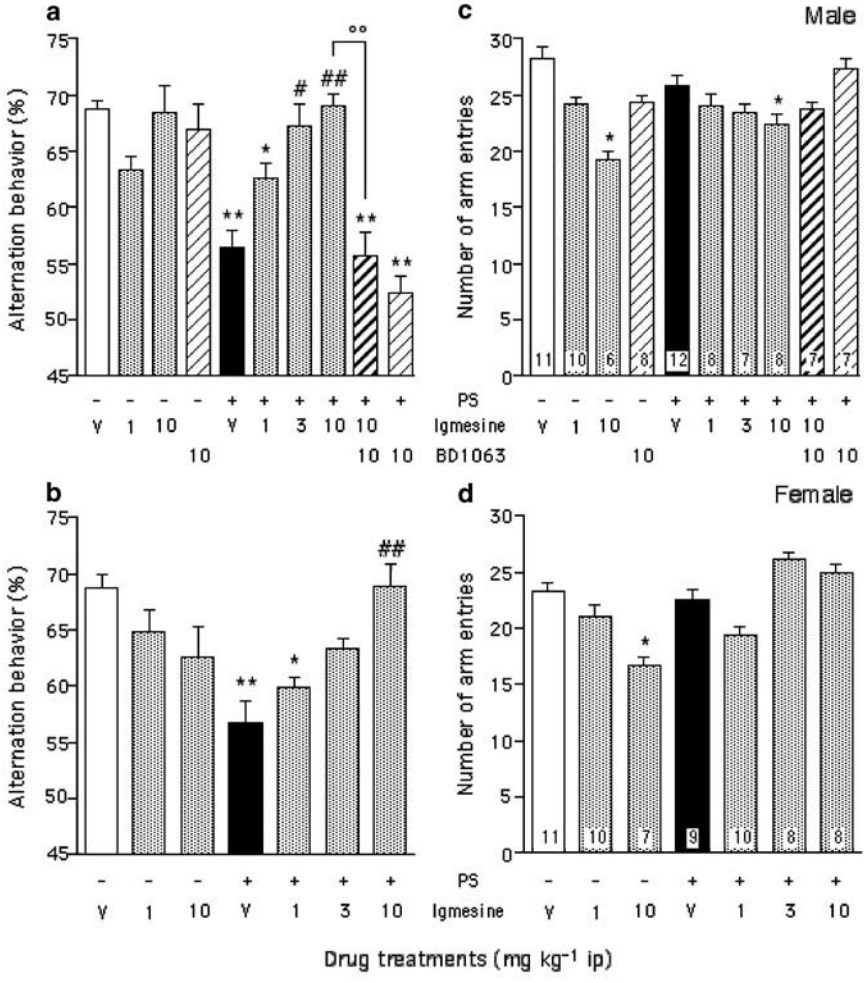

Figure 1 Effect of the σ_1 receptor agonist igmesine on the spontaneous alternation behavior deficits observed in prenatally stressed rats in the Y-maze test: alternation percentages (a, b) and total number of arm entries (c, d). Male (a, c) and female (b, d) rats subjected to prenatal stress (PS) were administered i.p. with vehicle solution (V), igmesine (1, 3, 10 mg kg^{-1}) and/or BD1063 (10 mg kg^{-1}) 20 min before the session. The number of animals per group is indicated within the columns in (c, d). $^{*}P<0.05$, $^{**}P<0.01$ vs **V-treated no stress group; # $P<0.05$, ## $P<0.01$ vs V-treated PS group; $^{\circ\circ}P<0.01$ vs igmesine (10 mg kg^{-1})-treated PS group in (a); Dunnett's test.

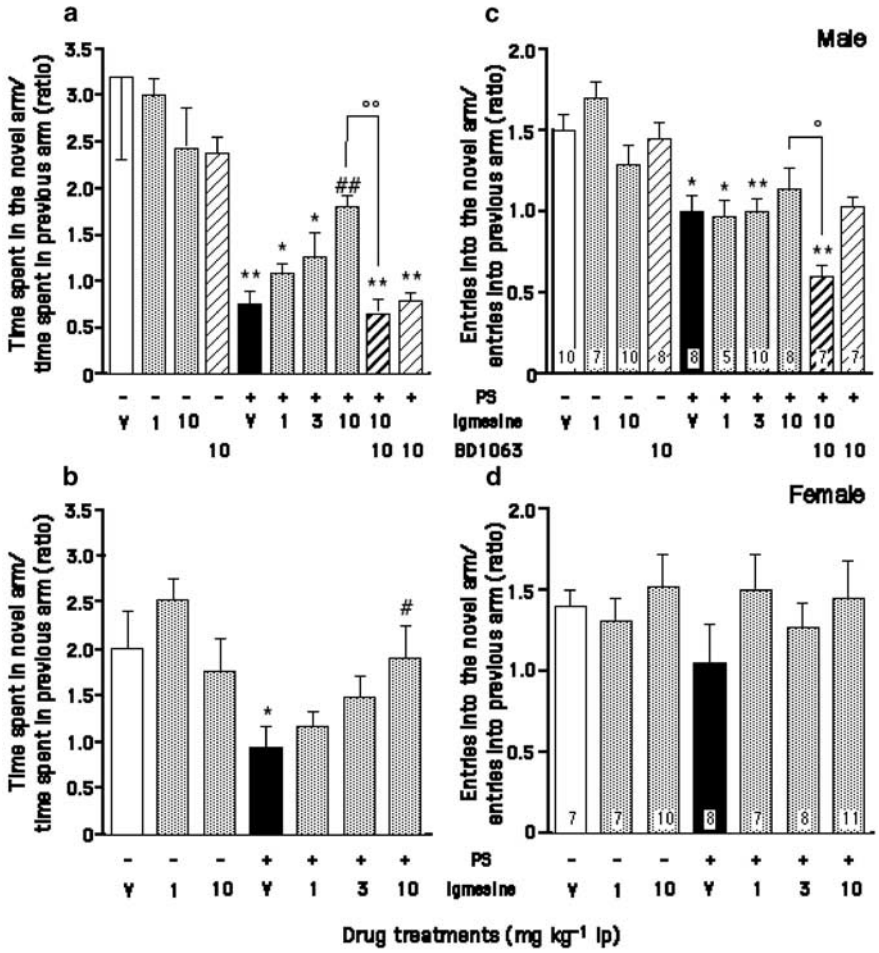

Figure 2 Effect of the σ_1 receptor agonist igmesine on the delayed alternation deficits in PS rats in the T-maze test: ratio of the time spent in the novel arm over the time spent in the previous arm (a, b) and ratio of the number of entries into the novel arm over entries into the previous arm (c, d). Male (a, c) and female (b, d) PS rats were examined separately. Animals were allowed to explore the T-maze, with one short arm closed, for 10 min. After 1 h time interval, the pattern of exploration of the whole maze was recorded during 2 min. Rats were administered i.p. with vehicle solution (V), igmesine (1, 3, 10 mg kg^{-1}) and/or BD1063 (10 mg kg^{-1}) 20 min before the second session. The number of animals per group is indicated within the columns in (c, d). *$P<0.05$, **$P<0.01$ vs V-treated no stress group; #$P<0.05$, ##$P<0.01$ vs V-treated no stress group; °$P<0.05$, °°$P<0.01$ vs igmesine (10 mg kg^{-1})-treated PS group; Dunnett's test.

animals spent more time in the novel arm: ratio = 3.2 for males (Figure 2a) and 2.0 for females (Figure 2b). PS resulted in the disappearance of this preferential exploration, with ratios close to unity. The treatment with igmesine (1–10 mg kg^{-1}) resulted in a dose-dependent reversion of the delayed alternation deficits, with no effect in non-PS rats. Indeed, both male and female offspring rats treated with the highest doses of compound (10 mg kg^{-1}) showed ratios significantly increased. The pretreatment with BD1063 failed to affect the performances of male non-PS or PS offspring rats, but highly significantly blocked the igmesine effect in PS rats (Figure 2a). Results analyzed in terms of number of visits led to significant differences only in males ($F(9,79)=6.10$, $P<0.0001$; Figure 2c) but not females ($F(6,57)=0.59$, $P>0.05$; Figure 2d). *Post hoc* comparisons showed that both male and female control rats visited significantly more frequently the novel arm, with ratios around 1.5 for males and 1.4 for females (Figure 2c and d), and PS resulted in a decrease of the ratios, close to unity, significantly in male but nonsignificantly in females. In males, the treatment with igmesine (10 mg kg^{-1}) attenuated the PS-induced decrease, but in a nonsignificant manner as compared with the vehicle-treated PS group (Figure 2c). The pretreatment with BD1063 however significantly blocked the igmesine effect (Figure 2c).

Place learning in the water-maze test

During days P28 to P32, offspring rats were trained to locate a fixed platform position in the water-maze. As shown in Figure 3, acquisition profiles did not differ among treatment groups for both male and female offspring. For the nonstressed vehicle-treated male rats (open squares, Figure 3a), the latencies to finding the platform decreased over the course of acquisition training ($Fr(4,49)=31.8$, $P<0.0001$). Between trials, there was a significant diminution of latencies between trial 1 and trials 4 and 5 ($P<0.01$). For the PS vehicle-treated group (open circles, Figure 3b), the latencies also decreased over the course of acquisition training ($Fr(4,49)=25.3$, $P<0.0001$). Between trials, there was a significant diminution of latencies between trial 1 and trials 3

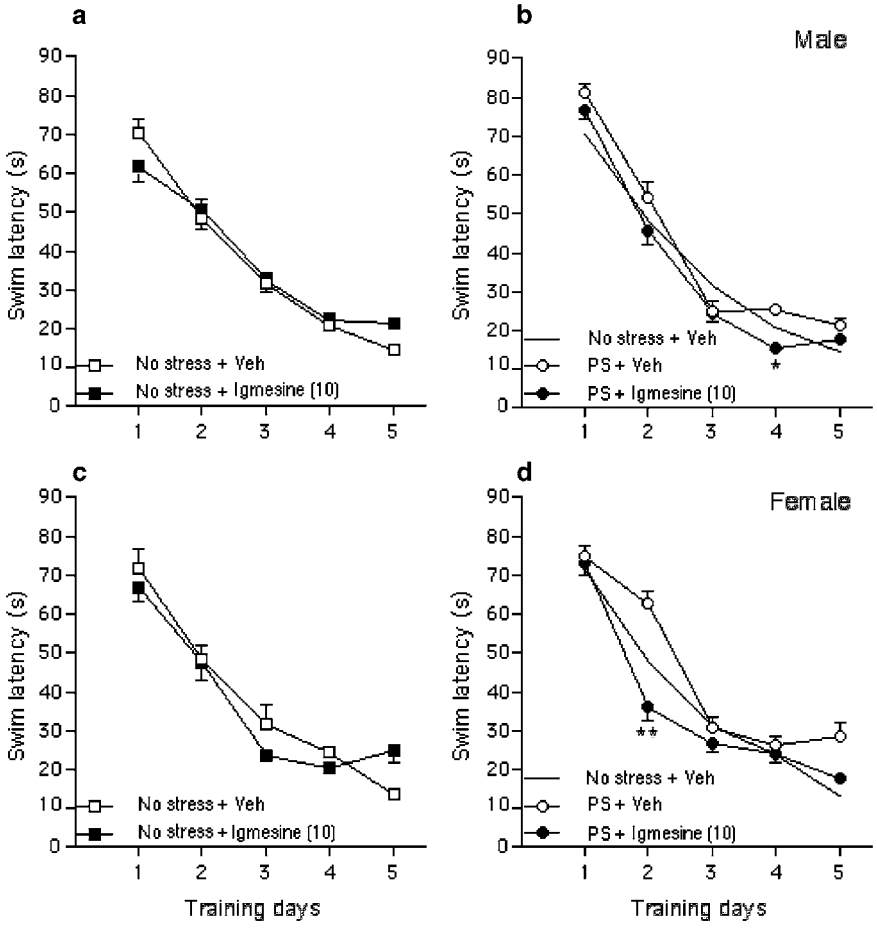

Figure 3 Acquisition profiles of nonstressed (a, c) or PS (b, d) rats for place learning in the water-maze: male (a, b) or female (c, d) rats. Animals were administered i.p. with vehicle solution (V), igmesine (1, 3, 10 mg kg^{-1}) and/or BD1063 (10 mg kg^{-1}) 20 min before the first trial and submitted during 5 days to three swims per day, with ITI of 10 min. The figures show acquisition profiles for Veh- and igmesine (10 mg kg^{-1})-treated groups only. In (b, d), the profile of the control (no stress + Veh) group is added as a simple line. The number of animals per group was $n = 7$–12 and 8–10 for the profiles shown in the figure. *$P<0.05$, **$P<0.01$ vs latencies shown by the vehicle-treated PS group during the same training day; Dunn's test.

($P<0.01$), 4 ($P<0.05$) and 5 ($P<0.001$). Latencies measured for each training day did not differ between nonstressed and PS groups ($P>0.05$ each). The treatments with the different doses of igmesine, or the BD1063 + igmesine combination, failed to affect the acquisition profiles for both nonstressed and PS animals, as shown for the 10 mg kg^{-1} dose in Figure 3a and b, with the exception of the latencies measured during trial 4 for PS rats (Figure 3b).

In female groups (Figure 3c and d), similar results were obtained. For the nonstressed vehicle-treated group (Figure 3c), the latencies to finding the platform decreased over the course of acquisition training ($Fr(4,39) = 18.6$, $P<0.001$). Between trials, there was a significant diminution of latencies between trial 1 and trial 5 ($P<0.001$). For the PS vehicle-treated group (Figure 3d), the latencies also decreased over the course of acquisition training ($Fr(4,44) = 22.0$, $P<0.001$). Between trials, there was a significant diminution of latencies between trial 1 and trials 4 ($P<0.01$) and 5 ($P<0.05$). Latencies measured for each training days did not differ between nonstressed and PS groups ($P>0.05$ each) and with the performances of male groups ($P>0.05$ each). The treatments with the different doses of igmesine failed to affect the acquisition profiles for both nonstressed and PS female rats (shown in Figure 3c and d for the 10 mg kg^{-1} dose), with the exception of the latencies measured during trial 2 for PS rats (Figure 3d).

During the probe test, performed 1 h after the last training session, significant differences were observed between nonstressed and PS groups, for both male and female offspring rats (Figure 4). The nonstressed vehicle-treated male rats swam preferentially in the T quadrant during the 60 s session ($F(3,39) = 17.6$, $P<0.0001$; Figure 4a). The time spent in this quadrant appeared highly significantly greater than the time spent in the other three, indicating that animals learned the invisible platform location correctly. PS vehicle-treated male rats also showed a differential pattern of exploration between each quadrant ($F(3,39) = 7.14$, $P<0.01$; Figure 4a). However, the time spent in the AR quadrant was not significantly different from the time spent in the T quadrant, indicating that learning of the visible platform location was more approximate. In addition, PS animals showed a highly significant diminution of the time spent in the T quadrant as compared with nonstressed animals (Figure 4a).

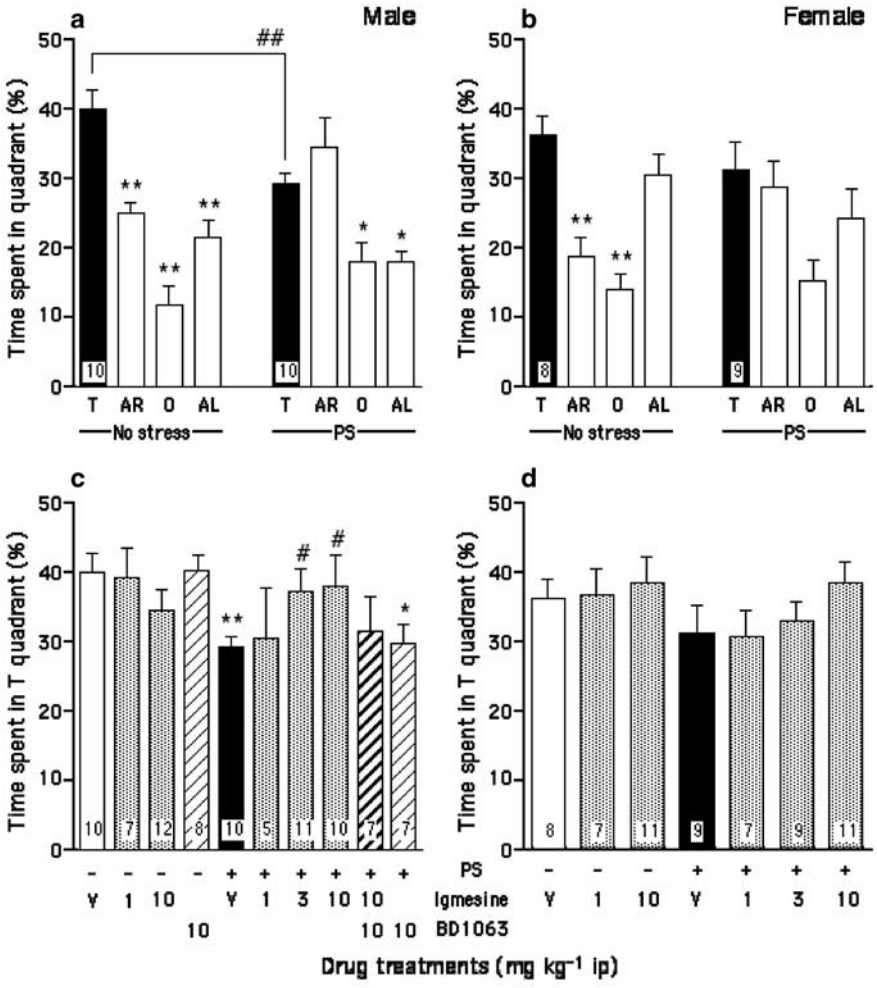

Figure 4 Performances of PS rats in the probe test for place learning in the water-maze: spatial preference among quadrants for male (a) and female (b) rats and effect of the σ_1 receptor agonist igmesine on the time spent in the training quadrant for male (c) and female (d) rats. At 1 h after the last swim trial, the platform was removed and animals were submitted to a 60-s swim. The presence in each quadrant was measured. Quadrants: T, training; AL, adjacent left; O, opposite; AR, adjacent right. The number of animals per group is indicated within the columns. $^{*}P<0.05$, $^{**}P<0.01$ vs time spent in the T quadrant; ## $P<0.01$ vs no stress group; Dunnett's test in (a, b). $^{**}P<0.01$ vs V-treated no stress group; # $P<0.05$ vs V-treated no stress group; Dunnett's test in (c, d).

The nonstressed vehicle-treated female rats also swam preferentially in the T quadrant during the 60 s session ($F(3,31)=10.8$, $P<0.001$; Figure 4b). The time spent in this quadrant appeared highly significantly greater than the time spent in the AR and O quadrants but not in the AL quadrant, suggesting that the spatial learning was in some extent rougher than for males. PS female rats showed a lack of preferential exploration among quadrants during the probe test ($F(3,35)=2.61$, $P>0.05$; Figure 4b). This observation suggested that, although the time spent in the T quadrant did not significantly differ between nonstressed and PS groups, PS resulted in marked place learning impairment in female offspring.

The effects of the different doses of igmesine on the time spent in the T quadrant are shown in Figure 4c for male and Figure 4d for female rats. Igmesine dose-dependently reversed the PS-induced diminution of exploration in the T quadrant for male rats ($F(9,86)=4.25$, $P<0.01$), with a significant effect measured for the 3 and 10 mg kg^{-1} doses (Figure 4c). The co-treatment with BD1063 blocked the beneficial effect induced by the highest dose of igmesine. In females, there was no significant overall effect ($F(6,61)=0.93$, $P>0.05$), but a tendency for a diminished time spent in the T quadrant for PS rats, dose-dependently attenuated by the igmesine treatment.

During days P33 to P35, animals were trained to learn a daily changing platform position. Swim performances were averaged over days for each swim trial as presented in Figure 5a and b for male and female offspring rats. Male nonstressed vehicle-treated rats showed a significant decrease over swim trials ($Fr(3,39)=25.7$, $P<0.0001$; Figure 5a). The first trial corresponded to the first exploration of the new platform location and can be considered as a sample trial. The latency to find the platform was significantly higher than the latencies measured for the three following trials ($P<0.001$). The marked decrease observed between trials 1 and 2 indicated that rats could rapidly summon up their working memory to locate efficiently the new platform location. Male PS vehicle-treated

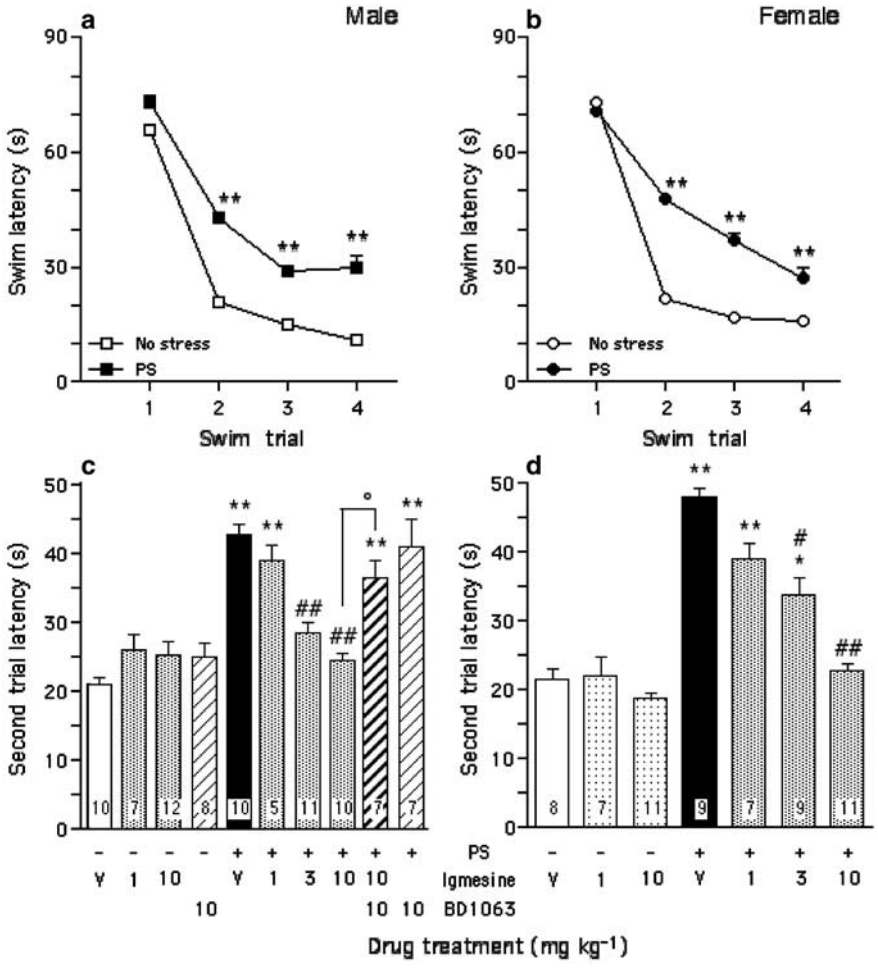

Figure 5 Working memory deficits observed in the water-maze effect for PS rats and beneficial effect of the σ_1 receptor agonist igmesine. Swim latency profiles among the four trials for nonstressed and PS rats (a, b). Effect of the σ_1 receptor agonist igmesine on the second trial latency (c, d) for male (a, c) and female (b, d) rats. The number of animals per group is indicated within the columns in (c, d). $^{**}P<0.01$ vs the same trial latency in no stress group; Mann–Whitney's test in (a, b). $^{*}P<0.05$, $^{**}P<0.01$ vs V-treated no stress group; # $P<0.05$, ## $P<0.01$ vs V-treated PS group; $^{\circ}P<0.05$ vs igmesine (10 mg kg^{-1})-treated PS group; Dunn's test in (c, d).

rats also showed a significant decrease over swim trial ($Fr(3,39)=21.1$, $P<0.0001$; Figure 5a). However, the latencies measured for the trials 2–4 were highly significantly higher than the latencies measured for the nonstressed rats (Figure 5a). Female nonstressed vehicle-treated rats showed a significant decrease over trials ($Fr(3,31)=16.4$, $P<0.001$; Figure 5b). The latency to find the platform was significantly higher than the latencies measured for the three following trials ($P<0.001$). Female PS vehicle-treated rats also showed a significant decrease over swim trial ($Fr(3,35)=21.5$, $P<0.0001$; Figure 5b). However, the latencies measured for the trials 2–4 were highly significantly higher than the latencies measured for the nonstressed rats (Figure 5b). In turn, marked impairments of the spatial working memory component were measured for both male and female PS rats. The spatial working memory could be assessed as the mean performance in the second trial for the 3 consecutive days. The effect of the igmesine, or igmesine + BD1063 combination, on the trial 2 latency is shown in Figure 5c and d for male and female rats respectively. In males, igmesine dose-dependently reversed the PS-induced increase in trial 2 latency ($F(9,86)=4.28$, $P<0.001$), with a significant effect measured for the 3 and 10 mg kg^{-1} doses (Figure 5c). BD1063 failed to affect the performances of non-PS or PS rats, but the co-treatment with BD1063 significantly blocked the beneficial effect induced by the highest dose of igmesine. In females, igmesine also dose-dependently reversed the PS-induced increase in trial 2 latency ($F(6,61)=11.58$, $P<0.0001$), with a significant effect at 3 and 10 mg kg^{-1} (Figure 5d).

Passive avoidance response

On day 36, offspring rats were trained in the step-through passive avoidance procedure. Retention session was performed after 24 h, and the latency to enter the darkened compartment as well as the percentage of animals reaching the avoidance criterion was determined (Figure 6). Nonparametric analyses of step-through latencies showed significant differences for male ($KW=16.23$, $P<0.05$; Figure 6a) and female ($KW=15.05$, $P<0.05$; Figure 6b) offspring rats. Indeed, both male and female PS rats presented a significant decrease of the step-through latency and the treatment with igmesine resulted

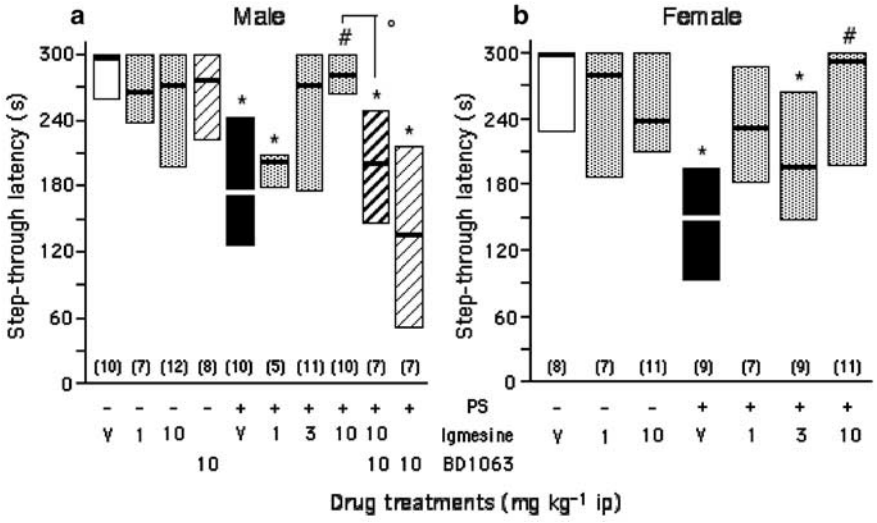

Figure 6 Effect of the σ_1 receptor agonist igmesine on the passive avoidance deficits in PS rats: step-through latencies showed by male (a) and female (b) rats, during the retention session. Rats were administered i.p. with vehicle solution (V), igmesine (1, 3, 10 mg kg^{-1}) and/or BD1063 (10 mg kg^{-1}) 20 min before the training session. After 24 h, they were placed into the white compartment and the latency was recorded up to 300 s. The number of animals per group is indicated between parantheses. *$P<0.05$ vs V-treated no stress group; # $P<0.05$ vs V-treated PS group; °$P<0.05$ vs igmesine (10 mg kg^{-1})-treated PS group; Dunn's test.

in a dose-dependent reversal of the long-term memory deficit, significantly at the highest dose tested (Figure 6a and b). In males, the BD1063 pretreatment significantly blocked the igmesine effect (Figure 6a).

Discussion

In the present study, we report that a selective σ_1 receptor agonist, igmesine, allows to fully reverse the learning impairments observed in PS rats. We first confirm that a PS, endured during the last week of gestation, leads to marked learning and memory deficits in the juvenile offspring. A semirandomized 90-min duration restraint stress applied during 4 days fails to affect the general maternal and offspring outcomes. However, significant learning and memory impairments are detected for both male and female 4-weeks old rats. PS-induced learning and memory deficits are known to persist throughout adulthood. For instance, adult PS rats show deficits in appetitive operant learning test, which appear mainly during the reversal stage of the operant discrimination task, with no effect on acquisition, discrimination or extinction (Weller *et al*., 1988). Adult PS rats, mainly the male but not female offspring, spent more time searching for the platform location in a water-maze task, when the water is kept at 12°C (Szuran *et al*., 1994). This observation was recently extended by Nishio *et al*. (2001) who reported that rats, prenatally stressed using a sound stress, exhibit an increased in total number of errors in the water-maze and time spent searching for the platform location. Lordi *et al*. (1997) also previously described that adult PS rats are impaired for delayed alternation behavior, with an ITI of 1 h but not with an ITI of 30 s or 3 min, and in a passive avoidance response. Finally, PS rats show an enhancement of the age (16–22 month)-related recognition memory impairment in a Y-maze procedure and the age-related working memory impairment in the radial-arm maze, as compared to nonstressed offspring rats (Vallée *et al*., 1999).

The learning and memory deficits observed in PS offspring are a consequence of developmental disturbances affecting neurotransmission systems. Indeed, forebrain cholinergic systems are affected and adult PS rats show increased hippocampal acetylcholine release after a mild stress or CRF administration (Day *et al*., 1998). PS rats also show reduced norepinephrine contents and increased norepinephrine turnover in the hippocampus and cortex (Huttenen, 1971; Takahashi *et al*., 1992). In addition, dopamine D2-like receptors are increased in dorsal frontal cortex, medial prefrontal cortex, hippocampal CA1 region and core region of nucleus accumbens of PS rats compared to control subjects (Berger *et al*., 2002). PS also affects GABAergic or glutamatergic systems. A decrease of hippocampal benzodiazepine receptors was measured (Fride *et al*., 1985; Takahashi *et al*., 1992), which may explain the high anxiogenic response observed in PS rats. Glutamate NMDA receptors are increased in medial prefrontal cortex, dorsal frontal cortex, hippocampal CA1, medial caudate-putamen, as well as in shell and core regions of nucleus accumbens (Berger *et al*., 2002). Group III metabotropic glutamate receptors were only found to be increased in medial prefrontal cortex and dorsal frontal cortex of PS rats (Berger *et al*., 2002). PS has also been reported to affect hippocampal corticosteroid receptors. Both glucocorticoid type I and II receptors are reduced in the hippocampus after PS, in relation with the HPA axis hyperactivity (Maccari *et al*., 1995). This decrease could be reversed by postnatal adoption, suggesting that environmental factors are sufficient to allow attenuation of the developmental deficits (Maccari *et al*., 1995). PS thus results in deficits affecting several neurotransmission systems, with the hippocampal formation appearing as a particularly vulnerable brain region. In turn, deleterious consequences are observed not only on the response to stress but also in terms of learning and memory abilities of the offspring. Interestingly, although most of the neurochemical analyses were performed in adult PS animals, the present behavioral

study suggests investigating neurochemical systems earlier in the lifetime.

Noteworthy, sex-related differences are inconsistently reported among studies. Szuran *et al.* (1994) reported that PS rats submitted to place learning in the water-maze fail to show any deficit when the water temperature was kept at 18°C, while female, but not male, PS rats show significant deficits when the water was cooled to 12°C. Nishio *et al.* (2001) reported that a maternal sound stress combined with a forced swimming stress potentiates the sound-induced loss of locomotor activity in male, but not female, PS offspring rats. In addition, an increase in the number of errors in the water-maze is measured only for male PS rats (Nishio *et al.*, 2001). We previously reported that the diminution of passive avoidance response is more markedly measured in female PS rats than in males, where it fails to reach significance (Gué *et al.*, 2004). The important sex-related differences observed in the hormonal response to PS may in part be related to the discrepancies observed in memory tests, since most of them, including the water-maze or passive avoidance response, involve stressful stimuli. Indeed, PS females show a higher ACTH response to acute stress than female controls, while PS males show a faster recovery of stress-induced ACTH increase than control males (McCormick *et al.*, 1995). PS males have even been reported to present reduced corticosteroid receptors density in the cerebral cortex, while female PS rats show an increased density (McCormick *et al.*, 1995). Therefore, the behavioral performances of PS animals, and notably sex-related differences, may markedly depend upon stressful stimuli and emotional state of animals performing the behavioral examination. In the present study, a mild sex-related difference is observed throughout the behavioral examination suggesting that PS differently affected to some extent the male and female PS offspring.

The most important point of the present study is to demonstrate that acute or repeated administration of igmesine, a selective σ_1 receptor agonist, reverses the learning and memory deficits observed in PS offspring. The compound, which is selective for the σ_1 receptor and present only a moderate affinitity for the σ_2 sites $-K_i(\sigma_1) = 39\,nM$, $K_i(\sigma_2) = 390\,nM$, ratio = 10 (Roman *et al.*, 1990) – is active in the 3–10 mg kg^{-1} dose range and significantly alleviates the delayed behavioral impairments, when administered acutely before the behavioral sessions. It appeared active in all aspects of the memory process, that is, spatial and nonspatial, short- and long-term memory components. Blockade of the igmesine pharmacological effect by BD1063 was systematically investigated in each test for male offspring. The compound is a selective σ_1 receptor antagonist $-K_i(\sigma_1) = 9\,nM$, $K_i(\sigma_2) = 449\,nM$, ratio = 50 (McCracken *et al.*, 1999). The blockade by BD1063 demonstrated the selective involvement of the σ_1 receptor in the behavioral response to igmesine.

The σ_1 receptors constitute important intracellular proteins, present in neurons from the embryonic developmental stage to aging, when they show a remarkable preservation of expression and anatomical distribution (Phan *et al.*, 2003). Unpublished observation of our team even revealed that the immunohistochemical distribution in adult is found similar to that observed at P15. Activation of the σ_1 receptor results in an efficient regulation of intracellular Ca^{2+} concentration (Su & Hayashi, 2003), resulting rapidly in both the neuromodulation of several responses to neurotransmitters, directly at the receptor activation level or indirectly through amplification of signal transduction systems (Monnet *et al.*, 1992; Gonzalez-Alvear & Werling, 1994; Su & Hayashi, 2003) and to neuroprotection (Nakazawa *et al.*, 1998; Maurice *et al.*, 1999b). At the behavioral level, no σ_1 receptor agonist was ever reported to present promemory ability in control animals, even submitted to a moderately reinforced learning task. However, acutely or repeatedly administered σ_1 compounds present antiamnesic properties in a variety of amnesia models. Pharmacological models, involving systemic administration of a selective receptor antagonist, including the NMDA receptor antagonist dizocilpine or the muscarinic acetylcholine receptor antagonist scopolamine, are attenuated by σ_1 ligands (for reviews, see, Maurice *et al.*, 1999b; 2001). Moreover, σ_1 receptor agonists are also efficient in pathological models of amnesia. Learning deficits observed after brain lesions could be alleviated by σ_1 receptor agonists, as showed in rats with basal forebrain lesion (Senda *et al.*, 1998), in mice exposed to carbon monoxide gas (Maurice *et al.*, 1994; 1999a) or intoxicated with trimethyltin (Maurice *et al.*, 1999a). In addition, selective σ_1 receptor agonists are also efficient in amnesia models related to normal or pathological aging, as demonstrated in aged rats and mice (Maurice, 2001; Tottori *et al.*, 2002; Phan *et al.*, 2003), SAM mice (Maurice *et al.*, 1996) or mice administered centrally with β-amyloid related peptide (Maurice *et al.*, 1998). The present study brings the first demonstration that σ_1 receptor agonists could also alleviate amnesia related to developmental disorders. PS-induced learning and memory deficits are fully reversed by igmesine, with the same efficacy in both male and female offspring.

The pharmacological action of the σ_1 receptor agonist may involve different, and putatively concomitant, mechanisms. First, activation of the σ_1 receptor by a selective agonist leads to the facilitation of responses to several neurotransmitters that are affected after PS. Within the hippocampal formation, a brain structure crucially involved in learning and memory processes, the σ_1 receptor activation results in the modulation of *N*-methyl-D-aspartate (NMDA)-type glutamatergic receptors, acetylcholine receptors or norepinephrine receptors (Maurice *et al.*, 1999b). Such effect has been recurrently evoked to explain the wide and effective antiamnesic activity of σ_1 receptor agonists. This hypothesis could be assessed within the hippocampus, for instance using an electrophysiological approach by measuring the modifications of long-term potentiation components induced after PS and igmesine treatment. Second, activation of the σ_1 receptor may result in direct effects on activation of the HPA axis, known to be hyper-responsive in PS rats. Indeed, σ_1 receptor ligands modulate the release of stress hormones. The σ_1 receptor antagonists (α-(4-fluorophenyl)-4-(5-fluoro-2-pyrimidinyl)-1-piperazine-butanol hydrochloride (BMY-148,02), rimcazole, or cis-*N*-cyclohexyl-*N*-ethyl-3-(3-chloro-4-cyclohexyl-phenyl)-propen-2-ylamine hydrochloride (SR 31747) increase plasma levels of corticosterone (Matheson *et al.*, 1991; Gudelsky & Nash, 1992; Derocq *et al.*, 1995; Eaton *et al.*, 1996), by inactivating norepinephrine neurons projecting to the paraventricular nucleus of the hypothalamus (Eaton *et al.*, 1996). Reciprocally, σ_1 receptor agonists, and particularly igmesine, inhibit not only several CRF-mediated stress responses such as gastric acid secretion or colonic spike burst frequency (Junien *et al.*, 1991; Gué *et al.*, 1992a, b), but also the anxiogenic behavioral response to CRF, in terms of number of entries and

time spent on the open arms of the elevated plus-maze (Song *et al*., 1997). In turn, the σ_1 receptor may be involved in a tonic negative regulation of the activity of the HPA axis, with antagonists facilitating the corticosterone release into the plasma, while agonists, through an anti-CRF effect, decreasing it. In addition, σ_1 receptor agonists, including (+)-SKF-10,047, (+)-pentazocine, (+)-3-[3-hydroxyphenyl-*N*(1-propyl)piperidine] ((+)-3PPP) or (−)-butaclamol, stimulate ACTH release after central or peripheral administration (Iyengar *et al*., 1990; 1991), and thus may mediate an indirect effect on glucocorticoid release from the cortico-adrenals. Consequently, activation of the σ_1 receptor may also contribute to the negative feedback action on the HPA axis. Therefore, σ_1 receptor agonists may regulate the responsiveness of the HPA axis in PS animals, sustaining the improving effects observed in behavioral responses.

Conclusion

The present study confirms that PS provokes marked learning impairments in the juvenile male and female offspring rat, affecting numerous aspects of the memory processes. Pretreatment with a selective σ_1 receptor agonist before each test session results in a complete reversal of the deficits. The extent of the σ_1 receptor-mediated beneficial action must now be characterized in its neurotransmission and neuroendocrine aspects.

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