Insulin-like growth factor 1 reduces age-related disorders induced by prenatal stress in female rats

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Abstract

Stress during the prenatal period can induce permanent abnormalities in adult life such as increased anxiety-like behavior and hyperactivity of hypothalamo-pituitary–adrenal (HPA) axis system. The present study was designed to investigate whether prenatal stress could induce spatial learning impairment in aged female rats. Furthermore, since it has been recently reported that insulin-like growth factor 1 (IGF-1) attenuates spatial learning deficits in aged rats and promotes neurogenesis in the hippocampus, we assessed the impact of a chronic infusion of IGF-1 on age-related disorders. Our results show that females stressed during prenatal life exhibit learning impairments in the water maze task. Chronic IGF-1 treatment restores their spatial abilities, reduces their HPA axis dysfunction and increases plasma estradiol levels. Parallel to these effects, chronic IGF-1 up-regulates neural proliferation in the dentate gyrus of the hippocampus. These findings support the hypothesis of an early programming of the vulnerability to some neurological diseases during senescence and reinforce the potential therapeutic interest of IGF-1 during brain aging.

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1. Introduction

Stressful events occurring during early life could increase vulnerability to the effects of stress later in life [19]. In rats, chronic stress during pregnancy exerts profound long-term influences on the offspring [33,54]. Prenatal stress induces an increase of the hypothalamo-pituitary–adrenal (HPA) axis activation in adult animals that is associated with a reduction in the number of hippocampal corticosteroid receptors [25,34]. This can be evidenced by a more prolonged elevation of plasma corticosterone after exposure to stress [34,51,55,56]. These HPA dysfunctions have been reported in young and aged animals, therefore suggesting a permanent effect of early stress [51]. In the brain, the main target of adrenal steroids is the hippocampal formation, which is involved in spatial memory processes [13]. Hippocampal neurons show remarkable plasticity, involving long-term potentiation, dendritic remodeling and neurogenesis, as well as a strong vulnerability to stressful experiences and to aging processes [20,38]. A recent study has provided evidence of a decrease of hippocampal neurogenesis after prenatal stress [28] and it was previously reported that prenatal stress increased age-related learning impairments [51]. Thus, HPA axis alterations by prenatal stress may be involved in the spatial memory impairments observed during aging, in agreement with the hypothesis of a “feed-forward” cascade whereby prolonged exposure to glucocorticoids damages the hippocampus and leads to cognitive deficits [45,46].

Extensive research suggests that exercise could have benefits for health and cognitive function particularly in aged individuals [9,11]. IGF-1 appears to play a major role in the effects of exercise on brain [6]. It regulates neurotrophic
response after injury [7], brain vasculature [47], brain glucose consumption [8], increases mRNA of brain-derived neurotrophic factor [6] and stimulates hippocampal neurogenesis [2,50]. Interestingly, aging is associated with reductions in the plasma and brain levels of IGF-1 [49].

Although considerable evidences show a link between stress, HPA axis dysfunction, memory disorders and aging, only one study has addressed the effect of prenatal stress on cognition in aged male rats [51] and nothing is known on the consequences of prenatal stress in aged females. Furthermore, females are known to be more vulnerable to stress [24] and exhibit an hyperactivity of the HPA axis function in comparison to male animals [57]. The aim of the present study was then to characterize the cognitive effect of prenatal stress in aged female rats and to determine whether IGF-1 could correct age-associated disorders. Therefore, we evaluated the spatial learning abilities of 24 month-old females that had been exposed to prenatal stress and we tested the effect of chronic infusions of IGF-1 on spatial performances, HPA axis function, estradiol levels and cell proliferation in the dentate gyrus.

2. Materials and methods

2.1. Animals and prenatal stress procedure

Sprague Dawley female rats were maintained on a 12:12 h dark:light cycle (lights on from 8 a.m. to 8 p.m.), with free access to food and water. Manipulation of the animals was performed following the principles of laboratory animal care published by the French Ethical Committee and the rules of the European Union Normative (86/609/EEC). Special care was taken to minimize animal suffering and to set the number of animals to the minimum required. During the last week of pregnancy, from day 14 until parturition, pregnant females were individually placed in plastic transparent cylinders (7 cm diameter, 19 cm long) and exposed to bright light for 45 min [25,34]. Animals were submitted to such three daily stress sessions (9 a.m., 12 p.m. and 5 p.m.), whereas control pregnant females were left undisturbed in their home cages. After weaning (21 days old), the offspring were housed in groups of four and left undisturbed. A maximum of two females by litter were used to avoid any “litter effect”. Animals with signs of respiratory distress or tumors were excluded. Two groups of 24 month-old animals were constituted: old control; old prenatally stressed. Moreover, a separate set of 2 animals was assigned to the young group.

2.2. Surgery

Animals were anesthetized using ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.). A 28-gauge steel cannula (Alzet-2004® brain infusion kit) was implanted into the right lateral ventricle (from bregma, anteroposterior: 0.8 mm, lateral: 1.5 mm; coordinates based on Paxinos and Watson [40]) and connected to an Alzet® osmotic minipump that was placed subcutaneously in the neck/shoulder region. Animals subjected to prenatal stress received either vehicle (NaCl) or recombinant human IGF-1 (GroPep, Australia), delivered at a rate of 50 ng per 0.25 μl per 21 days (Fig. 1). The remaining old rats as well as a group of 2-month-old young females served as controls and were infused with NaCl.

2.3. Water maze

The water maze task has been validated as a valuable index of spatial learning in aged rodents [14,30]. Apparatus consisted of a plastic tank, 2 m in diameter and 0.6 m in height. The tank was filled with water (22 ± 2 °C) to a depth of 35 cm [51,52]. The platform (10 cm diameter) was 2 cm above the surface of the water during the pretraining and 3 cm below the surface of the water during spatial learning. The pool, walls and platform were all colored black and indirect lighting was used in the room, enabling the platform to be hidden from sight. Extra-maze visual cues around the room remained in a fixed position throughout the experiment.

The timeline is illustrated in Fig. 1. Before spatial learning assessment, three sessions (consisting of three daily trials) of pretraining with a visible platform were conducted in order to train the rats to swim and climb onto the platform. This procedure allows to reduce the non-cognitive components of this task (stress reactivity, motor performances) and to control any difference between experimental groups in visual or motor abilities. Four days later, preoperative spatial learning performances were evaluated using a submerged platform (positioned in a different site from the pretraining). Three sessions were conducted, each consisting of three trials with
distinct start locations. If the rat did not find the platform in 90 s, it was guided to the platform. One week later, animals were implanted with an osmotic minipump and infused in the lateral cerebral ventricle (i.c.v.) for 11 days with saline or IGF-1. Animals were then tested in the water maze for 2 days. The procedure used was similar to the preoperative learning procedure, except that the hidden platform was localized at a different site. Latency to reach the platform was recorded by an automated system (Viewpoint, Lyon, France).

2.4. Behavior in the elevated plus maze test and corticosterone response

After 18 days of i.c.v. infusion of IGF-1 or vehicle, animals were exposed to the elevated plus maze to study anxiety-like behavior [41] (Fig. 1). The elevated plus maze was made of white wood and was 60 cm above the floor. It had four arms radiating outward from a central square (15 cm × 15 cm). Two were open (50 cm × 15 cm) and two were closed with side-walls (40 cm high). Each rat was placed on the central platform facing a closed arm, and allowed to freely explore the maze for 5 min. Exploration was recorded through an automated tracking system (Viewpoint, Lyon, France). The behavioral parameters scored were the number of entries into the closed and open arms and the time spent there. An entry was counted when the four paws were placed in the respective arm. Percentages of exploration of open arms (time and visits, ratio open/total arms) were calculated as an index of anxiety-like behavior.

Several studies have demonstrated an increase of corticosterone secretion after exposure to the elevated plus maze suggesting that this manipulation could be considered as stressful for the rat [27,44]. To determine plasma corticosterone after the plus maze exposure, blood samples (500 μl) were taken by cutting the tail (<2 min after removal from the plus-maze). Blood samples were then put into heparinized tubes, placed on ice, centrifuged and stored at −20 °C. The experiment was conducted between 10 a.m. and 1 p.m. to avoid the increase of plasma corticosterone induced by circadian rhythm.

2.5. 5-Bromo-2′-deoxyuridine injections and sacrifice

In the afternoon (5 p.m.) of the day 18 after the initiation of the i.c.v. infusion of NaCl or IGF-1, rats were injected with 5-bromo-2′-deoxyuridine (BrdU, 50 mg/kg, i.p.). Then, they received injections of BrdU twice per day (9 a.m., 5 p.m.) for 2 days and were killed between 15 and 20 h after the last injection of BrdU (Fig. 1).

Animals were deeply anaesthetized with pentobarbital and the cyclic status was determined by microscopic observation of vaginal smears. Adrenal glands were removed and weighed. Blood samples (500 μl) for plasma estrogen levels were taken by intracardiac punctures. Animals were then perfused intracardially with saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were removed and immersed in the same fixative for 4 h at 4 °C and then rinsed in phosphate buffer.

2.6. Immunohistochemistry

For each brain, coronal sections (50 μm thick) of the entire dentate gyrus of the right cerebral hemisphere were obtained with a Leica VIBrotome (Heidelberg, Germany). Endogenous peroxidase activity was quenched by incubating brain sections, for 30 min at room temperature, in 0.1 M phosphate buffer, with 10% methanol and 3% hydrogen peroxide. To ensure detection of BrdU-labeled nuclei, DNA was denatured by incubating the sections for 15 min at 37 °C, in 2 N HCl. After this step, sections were rinsed twice in 0.1 M boric acid buffer (pH 8.5), followed by a rinse in 0.1 M phosphate buffer, and then incubated for 1 h at room temperature, with 0.1 M phosphate buffer, 0.1 M, with 0.3% Triton X-100 and 0.3% bovine serum albumin. This buffer solution was used in the following washes and incubations. Sections were then incubated overnight, at 4 °C, with mouse anti-BrdU antibody (1:5000; Hybridoma Bank, Iowa City, IA). After rinsing in buffer, sections were incubated for 90 min at room temperature in biotinylated goat anti-mouse IgG (1:1000; Pierce, Rockford, IL), rinsed and transferred to the peroxidase avidin biotin complex (1:250; Pierce) for 45 min at room temperature. Peroxidase was detected using diaminobenzidine as chromogen.

2.7. Morphometric analysis of BrdU immunoreactive cells

The morphometric analysis of BrdU labeled cells was performed on coded sections. For each animal, BrdU positive cells were counted on every sixth section (300 μm apart) throughout the rostral-septal half of the dentate gyrus (from the rostral extreme of the hippocampus, at −1.80 mm from bregma, to the caudal end, at −6.80 mm from bregma). The same areas and number of sections were studied for all the animals and all the experimental groups. Sixteen sections were analyzed from each animal. All BrdU positive cells were counted with a 100× microscope objective. Cell counts were restricted to the granular cell layer (GCL) and the subgranular zone (SGZ) of the dentate gyrus. The SGZ was defined as a two-nucleus-wide band below the apparent border between GCL and the hilus. The total number of BrdU-labeled cells was estimated as previously described [4,23]. Briefly, BrdU-immunoreactive nuclei that came into focus while focusing down through the thickness of the section were counted, according to the optical dissector principle [10], whereas BrdU-immunoreactive nuclei located in the uppermost focal plane were ignored. We considered as BrdU positive nuclei those completely filled with DAB product or fluorescent marker or showing patches of variable intensity. The number of BrdU-immunoreactive nuclei counted in the GCL/SGZ was multiplied by 6 (because every sixth section was used) to estimate
the total number of BrdU-immunoreactive cells in the hippocampus.

2.8. Corticosterone and estrogen assays

Plasma corticosterone and estrogen levels were measured with radioimmunoassay kits (corticosterone kit: ICN, Biomedical, Orsay, France; estrogen kit: Diagnostic Products Corporation, Los Angeles, CA). The minimum levels of detection were 0.2 μg/dl for corticosterone and 1.4 pg/ml for estradiol. Intra-assay and inter-assay coefficients of variation were, respectively, 7 and 8%, for estradiol and 4 and 8%, for corticosterone.

2.9. Statistics

Initial spatial learning performances were assessed by two-way analysis of variance (ANOVA) using group as between factor (three levels) and sessions as within factor (three levels). Learning performances after surgery, endocrine data and cell proliferation in the dentate gyrus were analyzed with one way ANOVA. When significant, ANOVAs were followed by planned comparisons with contrast analysis for specific comparisons. Comparison of the percentages of memory-impaired rats in each group, before and after treatment, was assessed with t-test for percentages comparison. Correlations were calculated using Pearson’s test. Comparisons of the estrous cycle among groups were analyzed with a non-parametric test ($\chi^2$). Differences were considered significant at $P = 0.05$.

3. Results

3.1. Prenatal stress affects spatial learning in old females

Visual and sensorimotor capacities were assessed after 3 days of pretraining. The mean latency covered to find the visible platform was similar across groups (young: 15 s ± 2.4; old C: 15 s ± 1.3; old PS: 18 s ± 4; $F(2,37) = 0.59$, NS). As depicted in Fig. 2, latencies to locate hidden platform decreased across the testing sessions showing that all groups learned (session effect, $F(2,74) = 24.68$, $P < 0.0001$). Spatial learning capacities differed between groups throughout the 3 days of learning (group effect, $F(2,37) = 7.26$, $P < 0.002$). Planned comparisons by contrast analysis revealed that old rats subjected to prenatal stress exhibited an increased latency to find the submerged platform in comparison to old controls or young rats (PS versus C, $F(1,37) = 7.86$, $P < 0.01$; PS versus young, $F(1,37) = 12.25$, $P = 0.001$). In contrast, old C and young rats showed similar performances ($F(1,37) = 1.46$, NS).

Correlative analysis conducted on old animals (limited to rats treated with vehicle) revealed a significant positive correlation between spatial learning impairment and the weight of adrenal glands. Indeed animals that exhibited poor performances, i.e. the ones with higher latencies (mean over the 3 days of testing) to find the hidden platform, had higher adrenal gland weights ($r = 0.64$, $P < 0.01$). In contrast, no significant correlation was observed between the ability to reach the visible platform and the weight of adrenal glands ($r = 0.30$, NS).

3.2. IGF-1 improves spatial performances in old females subjected to prenatal stress

Given that only the old females that were subjected to prenatal stress exhibited memory impairments compared to young animals, the assessment of the influence of chronic infusions of IGF-1 on spatial learning was limited to this group. The differences in spatial learning abilities across the groups, described above (Fig. 2), were also observed by comparing the spatial learning performances before chronic i.c.v. infusion (group effect, $F(3,26) = 4.09$, $P < 0.05$). Before IGF-1 treatment, PS-IGF-1 and PS-NaCl groups exhibited an impaired spatial memory compared to C-NaCl animals (latency, $F(1,26) = 9.47$, $P < 0.01$) and compared to young females (latency, $F(1,26) = 4.97$, $P = 0.03$). After 11 days of i.c.v. treatment, a new spatial learning test was conducted. There was no significant decrease of the latency to reach the hidden platform throughout the two sessions of the second learning (session effect, $F(1,26) = 3.23$, NS).
However, as shown in Fig. 3A, the global performances differed across groups (group effect, F(3,26) = 3.74, P < 0.05). Planned comparisons indicated that learning performances were impaired in old PS-NaCl females as shown by the increased latency to reach the hidden platform in comparison with C-NaCl (F(1,26) = 8.32, P = 0.01). Subsequent analysis with young animals showed that the latency to reach the platform was similar across the groups, except for the old PS-NaCl females (young versus PS-NaCl, F(1,26) = 7.42, P < 0.01). Moreover, old PS females treated with IGF-1 exhibited an improvement of their spatial learning performances that just miss significance (PS-IGF-1 versus PS-NaCl, latency, F(1,26) = 3.87, P = 0.059). The percentage of old animals with learning impairments was determined based on the mean latency of young rats during the first two sessions, before and after chronic i.c.v. treatment (Fig. 3B). Before i.c.v. treatment, the percentage of rats showing learning impairments was 13% in the control group and 67% in animals exposed to prenatal stress (P < 0.05). After chronic treatment with IGF-1, this percentage was markedly decreased and reached the value of control females (after i.c.v. treatment, PS-NaCl versus PS-IGF-1 and C-NaCl, P < 0.05; PS-IGF-1 before treatment versus PS-IGF-1 after treatment, P < 0.05).

3.3. IGF-1 effects on anxiety, HPA axis function and estrogen levels in old females subjected to prenatal stress

In old rats, prenatal stress did not affect the percentages of time spent in the open arms (C-NaCl = 1.84 ± 0.91%, PS-NaCl = 6.78 ± 2.85%, PS-IGF-1 = 3.96 ± 1.23%) or the number of visits (C-NaCl = 22.78 ± 4.73%, PS-NaCl = 28.12 ± 7.83%, PS-IGF-1 = 34.60 ± 7.03) whatever the treatment (time spent, F(2,19) = 2.12, NS; visits, F(2,19) = 0.69, NS). Young animals spent more time than old animals in the open arms (young = 17.41 ± 5.05%, old = 3.96 ± 1.00%, F(1,28) = 15.76, P < 0.001) whereas no significant difference was observed for the number of visits (young = 34.47 ± 6.13%, old = 28.53 ± 4.29%; F(1,28) = 0.55, NS).

After a plus maze exposure, plasma corticosterone levels differed across the groups (F(3,24) = 3.95, P < 0.05). Prenatal stress increased the corticosterone response (PS-NaCl versus C-NaCl, F(1,24) = 11.52, P < 0.01), whereas IGF-1 treatment appeared to attenuate this effect (PS-IGF-1 versus C-NaCl, F(1,24) = 2.36, NS) (Fig. 4A). The increase in corticosterone secretion induced by the exposure to the plus-maze test was not affected by aging (young = 73.94 ± 8.17 μg/dl, old = 69.52 ± 3.8 μg/dl, age effect, F(1,26) = 0.24, NS). A significant group effect was also observed for the weight of adrenal glands (F(3,26) = 11.10, P < 0.001). PS-NaCl exhibited higher adrenal gland weights than C-NaCl (F(1,26) = 4.19, P < 0.05). Infusion with IGF-1 reversed this effect (PS-IGF-1 versus PS-NaCl, F(1,26) = 4.45, P < 0.05) as demonstrated by the similar adrenal weights observed in PS-IGF-1 and C-NaCl groups (F(1,26) = 0.01, NS) (Fig. 4B). The weight of adrenals was decreased in old animals compared to young (young = 116.57 ± 4.54 mg/kg, old = 81.78 ± 3.62 mg/kg; age effect, F(1,28) = 24.15, P < 0.001). In old females, cyclic status was similar across groups (they mainly exhibited diestrus or estrus type of smear, $\chi^2 = 0.78$, d.f. = 2, NS). However, plasma estrogen levels differed between groups in aged animals (F(2,14) = 6.79, P < 0.01) with higher levels in PS-IGF-1 females compared to groups infused with NaCl (PS-IGF-1 versus C-NaCl, F(1,14) = 8.53, P < 0.01; PS-IGF-1 versus PS-NaCl, F(1,14) = 10.63, P < 0.01) (Fig. 4C).
Fig. 4. Effect of IGF-1 on endocrine parameters in old prenatally stressed females. (A) Plasma corticosterone secretion (means ± SEM, n = 6–8 per group) in response to 5 min of elevated plus maze exposure in old C-NaCl, PS-NaCl and PS-IGF-1 animals. Prenatal stress increased corticosterone response after plus-maze (\(** P < 0.01\), PS-NaCl versus C-NaCl), whereas after IGF-1 treatment PS group did not differ from C group. (B) Adrenal weight (mg/kg) of old C-NaCl, PS-NaCl and PS-IGF-1 (means ± SEM, n = 7–8 per group). Adrenal weight was increased by prenatal stress (\(* P < 0.05\), PS-NaCl versus C-NaCl), IGF-1 treatment reversed this effect (\(\# P < 0.05\), PS-NaCl versus PS-IGF-1). (C) Plasma estradiol levels (means ± SEM, n = 7–8 per group) in old C-NaCl, PS-NaCl and PS-IGF-1. Chronic IGF-1 treatment increased estradiol levels in prenatally stressed rats (** P < 0.01, PS-IGF-1 versus C-NaCl or PS-NaCl).

3.4. IGF-1 increases BrdU labeling in the dentate gyrus of old females subjected to prenatal stress

The number of BrdU-immunoreactive cells in the granular and subgranular layers of the dentate gyrus was drastically affected by aging (young, 6502 ± 2908; old, 483 ± 124; age effect, \(F(1,18) = 93.10, P < 0.001\)). However, in old animals, the number of BrdU-positive cells differed between groups (\(F(2,12) = 8.83, P < 0.01\)) and was increased after 21 days of IGF-1 infusion in PS old females (PS-IGF-1 versus C-NaCl, \(F(1,12) = 14.88, P < 0.01\); PS-IGF-1 versus PS-NaCl, \(F(1,12) = 11.38, P < 0.01\)) (Fig. 5). The number of BrdU-positive cells in the dentate gyrus of PS rats treated with IGF-1 showed a 2.5–3 time increment compared to C-NaCl or PS-NaCl groups.

The number of BrdU-labeled cells in the dentate gyrus and the weight of adrenal glands were negatively correlated in the...

Fig. 5. Effect of IGF-1 on the number of BrdU-labeled cells, in the granular and subgranular layers of the dentate gyrus, in old prenatally stressed females. (A) Quantification of BrdU-positive cells in the granular cell layer and subgranular zone (GCL/SGZ) of the dentate gyrus in old C-NaCl, PS-NaCl and PS-IGF-1 (means ± SEM, n = 7–8 per group). Chronic IGF-1 infusion increased the number of BrdU-positive cells of the PS group (** P < 0.01, in comparison with C-NaCl and PS-NaCl). Representative photomicrographs of BrdU-labeled cells in subgranular zone of the dentate gyrus from old females subjected to prenatal stress chronically infused with (B) NaCl or (C) IGF-1. Scale bar for (B) and (C): 100 μm. Insets represent a high magnification of a cluster of newborn cells showing BrdU immunoreactive cells. Scale bar for insets: 10 μm.
prenatally stressed animals (Pearson’s correlation; \( r = -0.73 \), \( P < 0.05 \)). The number of BrdU cell was also negatively correlated with corticosterone levels after stress in this experimental group (Pearson’s correlation; \( r = -0.89 \), \( P < 0.001 \)). No significant correlations were shown in the other experimental groups.

4. Discussion

Our results show that females stressed during prenatal life exhibit spatial learning impairments with aging. Chronic IGF-1 treatment restores their spatial abilities, reduces their HPA axis dysfunction and increases plasma estradiol levels. Parallel to these effects, chronic IGF-1 up-regulates neural proliferation in the dentate gyrus of the hippocampus. Although mechanisms underlying the effects of IGF-1 remain to be fully elucidated, our results suggest an “anti-aging” activity of this neurotrophin.

Among the factors involved in the vulnerability to aging-associated cognitive disorders, stress and glucocorticoids have been proposed to play a major role [38,39]. Here we demonstrate for the first time that spatial learning is impaired in 24 month-old females exposed to stress during the prenatal period. These results confirm and extend a previous work showing an impairment of spatial memory in a spontaneous recognition task in old male rats subjected to prenatal stress [51]. Our results demonstrate that chronic infusion of IGF-1 suppresses spatial learning deficits in cognitively-impaired old animals. Indeed, aged females subjected to prenatal stress, that exhibited a marked impairment in their learning abilities before treatment, reached the performances of old control and young females after i.c.v. infusion of IGF-1 for 11 days. This result reinforces previous findings showing an improvement in spatial reference memory and in object recognition in old male rats after chronic treatment with IGF-1 [36,49]. Water maze is a stressful task, especially with water at 22°C [32]. We have used water at 22°C based on previous studies conducted in aged animals in a similar paradigm [51,52]. This stressful situation allows maintaining high levels of motivation in aged animals and prevents behavioral strategies such as floating behavior often observed in these animals at higher temperatures. The lack of effect of IGF-1 on anxiety, observed in the elevated plus-maze, suggests that the enhancement of learning performance by IGF-1 in rats subjected to prenatal stress was not related to a secondary effect of IGF-1 such as a decrease of fearlessness in the water maze.

Old females subjected to prenatal stress exhibited higher corticosterone after plus-maze exposure and had increased adrenal gland weights according to previous reports in males [28,53]. Age-related changes of the HPA axis have been shown to be involved in cognitive aging. We observed a significant increase of adrenal function between memory impaired and memory non-impaired aged rats, but surprisingly not between old and young animals. The use of very young females (2 months old) may explain our results. In this view, adrenal hypertrophy with aging is only observed when old animals are compared to adult rats (6 months old) whose growth is essentially completed [58]. When compared to younger animals (28 days or 3 months old), old rats show lower adrenal gland weight [28]. Although extensive evidence indicates that prenatal stress induces HPA dysfunction in young rats [25,37-55], we show here for the first time that the effects of prenatal stress on the HPA axis may persist until senescence in females. The elevated plasma corticosterone levels indicate that hormonal response to a stressor is more pronounced in rats subjected to prenatal stress. A chronic activation of the adrenal glands in aged animals, resulting in heightened release of corticosterone, has been shown to be associated with hypertrophy of the adrenal glands due to the hyperstimulation by the pituitary [43]. Considering both control and prenatally stressed females, our results give evidence of a link between spatial learning and HPA axis dysfunction in aged animals. Indeed, old females with higher learning deficits in the water maze had higher adrenal gland weights. This observation strongly supports previous findings in animals and humans of a correlation between the hyperactivity of the HPA axis and the development of cognitive impairments with aging [22,26,31,42,59].

We observed that chronic i.c.v. infusion of IGF-1 attenuated HPA axis activity and increased plasma estradiol levels. The mechanisms underlying this effect remain to be elucidated. It could be hypothesized that central IGF-1 is involved in the feed back processes of HPA axis activity through a decrease of CRH and/or ACTH release or through a modulation of hippocampal glucocorticoid receptors and/or a modulation of neurotransmitters regulating this axis. Moreover, since the ovaries of old rats are capable of near normal function under appropriate gonadotropic stimulation [21], IGF-1 action could be mediated by a modulation of the hypothalamic-pituitary function and/or ovarian function, resulting in an increase in estradiol synthesis [12]. It is also possible that the increase in circulating estradiol was related to changes in the clearance of estradiol. The inhibition of HPA axis hyperactivity and/or the stimulation of estradiol secretion may be involved in the processes by which IGF-1 restored cognitive function in aged females. Indeed, chronic exposure to high levels of glucocorticoids are damaging for the hippocampus, inducing atrophy, synaptic loss and a decrease of cell proliferation [38]. In contrast, estrogen replacement improves spatial reference memory in aged female rodents [15,35] and stimulates cell proliferation in the hippocampus [3]. Furthermore estrogen and IGF-1 have been proposed to interact in the regulation of brain plasticity [5,17].

Our data indicate that chronic i.c.v. infusion of IGF-1 increases cell proliferation in the dentate gyrus of old female rats exposed to chronic stress during fetal life. This finding corroborates recent studies conducted in male rats showing that IGF-1 promotes hippocampal neurogenesis in young adults [1,50] and in old animals [29]. Recent data showing a correlation between spatial learning performances in the water maze and cell proliferation in the hippocampus [28]...
suggest that an increase of cell proliferation induced by IGF-1 may be important in its behavioral effect. Interestingly, we report here that the enhancement of cell proliferation induced by IGF-1 treatment was associated with an improvement of HPA axis function in the same animals. IGF-1 and adrenal steroids have been suggested to work antagonistically to regulate hippocampal neurogenesis. Exposure to stressful experiences, including early stress events, reduces cell proliferation [18,28,48]. However, although prenatal stress resulted in an increased weight of adrenal glands in old females, no differences were observed in hippocampal cell proliferation between prenatal-stressed and control old females. This observation is apparently in contrast with the recent finding showing a reduced neurogenesis in old male rats exposed in utero to the same chronic stress [28]. A gender difference could be taken into account to explain this discrepancy, since higher levels of estrogen and transcortin occurring in females throughout life could protect hippocampus. In this view, it has been shown that in comparison with male rats, females exposed to chronic restraint stress exhibited higher corticosterone levels but lower hippocampal atrophy [16].

In conclusion, our results demonstrate for the first time, that chronic IGF-1 treatment in aged females stressed during fetal life reduces spatial learning impairment in the water maze. Although additional work is necessary to unravel the mechanisms underlying the beneficial effect of IGF-1, it could be hypothesized that hormonal changes (reduced corticosterone and increased estradiol levels) induced by IGF-1 treatment may help to restore brain plasticity and/or neuroprotection of the hippocampus. These results support the interest of studying the effects of IGF-1 in age-related disorders and reinforce the hypothesis that early adverse events may have a profound impact on future adaptive abilities of an organism throughout his life-time, from adulthood to senescence.

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