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Neurobehavioural effects of exposure to fluoride in the earliest stages of rat development

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HIGHLIGHTS

- Exposure to low levels of Fluoride during pregnancy and lactation was studied.
- Fluoride produces a delay in eye opening development in all offspring.
- Adult offspring exposed to low Fluoride concentrations showed hypoactivity.
- Exposure to F reduced anxiety levels in young female and in all adult offspring.
- Low F concentrations produce dysfunction in the central nervous system.

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ABSTRACT

It is known that exposure to high concentrations of Fluoride (F) produces deleterious health effects in human population. However, in the last years it has been concluded that low concentrations of F may have adverse health effects as well. Transplacental passage of F and its incorporation into foetal tissues has been demonstrated. Therefore, the purpose of the present work was to study the effects of the exposure to low levels of F during pregnancy and lactation on the central nervous system functionality. Wistar rats were exposed to low F concentrations (5 and 10 mg/l) during pregnancy and lactation. Sensorimotor reflexes in the each pup were analysed and the postnatal day on which both eyes and auditory canals were opened was recorded. Locomotor activity and anxiety were subsequently analysed in 45- and 90-day-old offspring by an open field test and plus maze test, respectively. A significant delay in the development of eye opening was observed in all offspring whose mothers had been exposed to the two F concentrations tested. Exposure to 5 and 10 mg/l F was also found to significantly decrease locomotor activity only in 90-day-old male and female offspring. A low index of anxiety in the young females and in all adult offspring exposed to the two F concentrations tested was also detected. Taken together, findings from the present study show that exposure to low F concentrations during pregnancy and lactation produces dysfunction in the central nervous system mechanisms which regulate motor and sensitive development, locomotor activity and anxiety.

1. Introduction

Safe drinking water is the primary need of every human being. Although groundwater is considered safe it is contaminated with soluble organic and inorganic materials. Among common inorganic contaminants are fluoride (F), nitrates and nitrites and several heavy metals. Fluorides of several metals and nonmetals are important industrial chemicals and are mainly used for aluminium production, drinking water fluoridation and the manufacture of fluoridated dental preparations [1,2]. F is toxic when consumed in excess but of benefit to human health when consumed within the permissible limit [3]. The beneficial effects of F are achieved at low concentrations (0.8–1.2 mg/l, equivalent to doses of 0.02–0.03 mg/kg) in drinking water and when it is mixed with dental paste except that, in the latter case and at high concentrations (>1.5 mg/l), it produces dental fluorosis, the first visible toxic effect of F exposure [4], which manifests as pitting of tooth enamel and yellow cracked teeth in adults and in children.

Groundwater is naturally contaminated all over the world with F at levels ranging from 1 to more than 25 mg/l [5]. In Argentina, in

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particular, some areas of the Chaco-Pampean plain have been found to have shallow groundwater with high F concentrations (11.5 mg/l), this being a potential risk of fluorosis [6]. Fluorosis, an endemic public health problem in 22 countries, produces skeletal fluorosis, dental fluorosis, and non-skeletal fluorosis affecting soft tissues, such as muscles, liver, kidney and nervous system.

F can penetrate into the brain. Thus, at high concentrations as it is typically found in patients with fluorosis, F produces harmful effects to the brain. Epidemiological studies carried out since 2006 have documented F as a neurotoxicant [7]. F exposure increases the risk of low intelligence quotient of children grown up in F endemic areas in China [8]. Also, several surveys of persons chronically exposed to industrial F pollution reported symptoms related to impaired central nervous system functioning with decreased cognition and memory [9]. Locomotor impairment was also recorded in both sexes of children and adults [10]. F harmful effects on human beings were also observed in experimental animals (impaired locomotion, learning and memory process), thus indicating that animals' response to toxic F concentrations is similar to that of human beings [4].

Previous research has also demonstrated that in human beings as well as in rats F administered during gestation crosses the placenta and is also present in mother's milk [11]. In addition, F, which is biologically active even at the lowest concentrations, can readily penetrate into cell membranes by simple diffusion and cause adverse effects on cell metabolism and function. Exposure to NaF (50, 100 mg/kg) through drinking water during gestation has resulted in histopathological changes, such as alveolar cell hyperplasia and necrosis in the lungs of rats over several generations [12]. In addition, administration of NaF (40 mg/kg) in rats during pregnancy and lactation was observed to result in hypoproteinemia and hypoglycemia in mothers and offspring [13]. A decrease in uterine weight and foetus number was recorded in rats orally exposed to NaF (40 mg/kg) during days 6–19 of gestation [14]. In addition, impairment of motor coordination, learning and memory as a result of perinatal exposure to NaF (5 mg/kg) was found to be more marked in male than in female offspring [15]. In spite of all the above-listed studies, literature is poor on the effects on the central nervous system of the exposure to low F doses during pregnancy and lactation.

It is therefore hypothesized that F exposure during pregnancy and lactation could lead to structural alterations in the neuronal circuit which may later manifest as functional deficits. The purpose of the present work was to study the effects of the exposure to low levels of F during pregnancy and lactation on the central nervous system functionality. To this end, Wistar rats were exposed to low F concentrations (5 and 10 mg/l) during pregnancy and lactation and sensorimotor reflexes were analysed in the newborn. The postnatal day on which righting reflex, cliff aversion and negative geotaxis were reached and on which both eyes and auditory canals were opened was recorded for each pup in order to evaluate sensorimotor development. Both locomotor activity and anxiety were also analysed in 45- and 90-day-old offspring by means of an open field test and a plus maze test, respectively.

2. Materials and methods

2.1. Materials

Sodium fluoride (NaF) was purchased from Anedra (San Fernando, Argentina).

2.2. Animals

Parent animals were male and nulliparous female Wistar rats (90–120 days old) from our own breeding center. They were maintained under constant temperature (22° ± 1 °C) and humidity (50–60%) conditions in a 12 L:12D cycle (lights on at 7:00 h) and with food and water ad libitum. In the evening of the proestrus day, they were housed overnight with the male rats. The presence of spermatozoa in the vaginal smears was registered as an index of pregnancy and was referred to as gestational day 0 (GD 0). Pregnant females were weighted and housed individually in boxes and were randomly assigned to one of the three following groups: control group ($n = 10$), F treated group with 5 mg/l in drinking water ($n = 10$) and F treated group with 10 mg/l in drinking water ($n = 10$), equivalent to doses of 0.6 and 1.2 mg/kg, respectively. Drinking water was changed daily. Maternal weight gain and food intake were recorded on different gestational days (0, 3, 6, 9, 12, 15, 18 and 20) and postgestational days (1, 4, 7, 10, 13, 16, 19, and 21). Drink consumption was recorded daily. Within 24 h after delivery, all pups were weighted and litters were randomly culled to five males and five females whenever possible. Gestation length, litter size and body weight of pups at different postnatal days (1, 4, 7, 10, 13, 16, 19 and 21) were analysed. Offspring were weaned and housed in groups of six rats according to sex and treatment. One male and one female from each litter were used for the neurobehavioural tests.

2.3. Sensorimotor development

Starting on postnatal day 3, each pup received a battery of developmental tests. One test trial per day was given to the pups on each test. The dependent variable analysed for each test consisted in the postnatal day until the following criteria were reached by each pup.

2.3.1. Righting reflex

Each pup was placed on its back on a cloth-covered supporting surface and was allowed to right itself. This reflex was registered as mature if the pup performed this response within 5 s on 2 consecutive days.

2.3.2. Cliff aversion

Offspring were placed with their forepaws on the edge of a wooden platform and the snout protruded beyond the edge of the same platform. The latency to retract their body 1.5 cm from the edge was registered. The cliff aversion criterion was registered as mature when the pup performed this response in less than 5 s on 2 consecutive days.

2.3.3. Negative geotaxis

Each rat was placed on an inclined wire mesh ramp (angle of inclination from the base: 30°) with the head facing down. This criterion was registered as mature when pups reached a 180° rotation of the body and climbed upwards within 10 s on 2 consecutive days.

2.3.4. Eye and ear opening

The postnatal days on which both eyes were opened and on which both auditory canals were fully opened were registered.

2.4. Open field

The locomotor activity of all animals was analysed in an open field. This test was performed in 45- and 90-day-old offspring separately, both groups treated with 5 and 10 mg/l of F in drinking water during pregnancy and lactation. Each 45- and 90-day-old offspring was placed in an open area of 50 × 50 × 60 cm whose floor was divided into 12 × 12 cm squares by black lines. The number of squares entered by each rat with all four paws, rearings (occasions on which the animals stood on their hind legs), groomings (face washing, forepaw licking and head stroking) and faecal boluses were scored every 5 min for 15 min. The number of squares crossed and the rearings were recorded as parameters of locomotor activity, whereas the number of groomings and the number of faecal boluses deposited were considered as parameters of emotionality [16,17]. Once each animal was removed, the open field was carefully cleaned with a damp cloth.

2.5. Plus maze

This test was performed in 45- and 90-day-old offspring separately, both treated with 5 and 10 mg/l of F in drinking water during pregnancy and lactation.

Tests on the plus maze were conducted in a room with fluorescent lighting and minimal noise. The plus maze was made up of wood and consisted of four arms, all with the same dimensions (50 × 10 cm), and which were elevated 50 cm above the floor. Two of these arms were enclosed by 40 cm high lateral walls with an open roof, and were located perpendicularly to the other two opposed open arms. The four arms delimited a central area of 10 cm^2. In rodents, this test exploits the natural conflict between avoidance and exploration of open and elevated areas. Thus, rats were placed in the center of the maze facing an enclosed arm and were allowed to explore the maze freely for 5 min. The following parameters were estimated: i) percentage of time spent in the open arms, ii) percentage of entries into the open arms, and iii) total number of arm entries (which keeps record of locomotor activity). Increased time spent and number of entries into the open arms are consistent with a decrease in anxiety behaviour [18]. The floor of the maze was wiped thoroughly with damp tissue after each test.

2.6. Statistics

The data about mothers and their litters were analysed by one-way ANOVA and repeated-measures ANOVA. Each sensorimotor development test was analysed by two-way ANOVA (group × sex) followed by LSD test. The data obtained in the total 15 min of observation in the open field were analysed using a three-way ANOVA (group × sex × age) followed by post hoc comparisons using LSD test. For the comparative analysis of the parameters evaluated every 5 min, a repeated-measures ANOVA was used. In order to analyse the differences in each period of 5 min within each group, a *t*-test for paired samples was used, and a *t*-test for independent samples was carried out to analyse the differences in each period of 5 min between the groups tested.

Plus maze variables were analysed by three-way ANOVA (group × sex × age) and the differences between groups were assessed using LSD post hoc test. Probability values lower than 0.05 were considered to be significant. All statistical analyses were carried out using software SPSS 21.0 for Windows.

2.7. Ethics

Animal care and handling were in accordance with the internationally accepted standard Guide for the Care and Use of Laboratory Animals [19] as adopted and promulgated by the National Institute of Health. Experimental designs were also approved by the local standard for protecting animal's welfare, Institutional Committee for the Care and Use of Experimental Animals.

3. Results

3.1. Data about the mothers and their litters

Maternal weight gain and food intake were recorded on different gestational days, every 3 days, of the control and F-treated groups (5 and 10 mg/l). Drinking water consumption was recorded daily. All pups were weighted and gestation length, litter size and body weight of pups on different postnatal days were analysed. No statistical differences were recorded in body weight among the groups of dams on gestational day 0, nor in weight gain during all the different periods registered (days 3, 6, 9, 12, 15, 18 and 20), nor in gestational length and litter size (Table 1).

Also, F exposure during pregnancy and lactation did not significantly affect the body weight of female and male pups on different postnatal days (1, 4, 7, 10, 13, 16, 19 and 21; Fig. 1A). No visible teratogenic malformations were observed in any of the groups tested. Food and water intake during pregnancy (gestational) and lactation (postgestational) were not affected in the mothers exposed to the two F concentrations (5 and 10 mg/l) tested with respect to the control group (Fig. 1B and C). No statistical differences were observed among the different groups.

Table 1
Data of the mothers and their litters.

	Control group	5 mg/l F	10 mg/l F
	$n = 10$	$n = 10$	$n = 10$
Body weight of dams (g)			
GD 0	278.0 ± 13.2	270.4 ± 18.6	268.0 ± 12.9
Weight gain (g)			
GD 0–3	13.2 ± 1.2	16.0 ± 1.0	17.5 ± 0.9
GD 3–6	8.1 ± 1.6	6.4 ± 0.7	7.5 ± 1.6
GD 6–9	11.4 ± 1.9	14.3 ± 1.8	8.8 ± 1.8
GD 9–12	17.0 ± 1.7	11.7 ± 2.1	19.8 ± 2.0
GD 12–15	16.3 ± 2.1	23.6 ± 4.4	12.8 ± 3.2
GD 15–18	33.6 ± 3.0	35.0 ± 5.9	34.8 ± 4.9
GD 18–20	26.3 ± 2.2	29.6 ± 2.7	37.5 ± 7.6
GD 0–20	126.0 ± 4.5	136.6 ± 5.8	138.5 ± 7.4
Length of gestation (days)	22 ± 0	22 ± 0	22.3 ± 0.3
Litter size			
Female	5.8 ± 1.1	4.4 ± 0.9	9.3 ± 0.9
Male	6.0 ± 0.9	6.4 ± 0.6	5.0 ± 0.9
Total	11.7 ± 1.0	10.8 ± 1.4	14.3 ± 3.8

Values are mean ± SEM. GD (gestational day).

In conclusion, the F concentrations used did not affect the normal development of pregnancy and lactation.

3.2. Sensorimotor development

From the third postnatal day onwards, we analysed in each pup a battery of developmental tests that included righting reflex, cliff aversion, negative geotaxis and eye and ear opening. No statistical differences were observed among the different groups in righting reflex, cliff aversion and negative geotaxis (Table 2) between control and F-treated (5 and 10 mg/l) groups.

As to eye opening in offspring, two-way ANOVA revealed significant differences in groups, $F_{(2,54)} = 31.82$, $P < 0.001$. Post hoc comparison showed that gestational and lactation exposure to F produced a significant delay in the development of eye opening in female ($P < 0.001$ and $P < 0.01$, for 5 and 10 mg/l F, respectively) and male offspring ($P < 0.001$ and $P < 0.05$, for 5 and 10 mg/l F, respectively). No statistical differences were observed regarding the day of ear opening (Table 3) in the female and male offspring exposed to the two F concentrations tested.

Summing up, eye opening was the only sensorimotor parameter affected as a result of exposure to F, which was confirmed by a significant delay in eye opening time.

3.3. Open field

In the number of squares crossed by rats during a total period of 15 min, three-way ANOVA showed significant differences among groups, $F_{(2108)} = 19.06$, $P < 0.001$, sexes, $F_{(1108)} = 34.57$, $P < 0.001$, and ages, $F_{(1108)} = 4.15$, $P < 0.05$. It also revealed significant differences in the interaction between factors: group × age, $F_{(2108)} = 28.34$, $P < 0.001$, and sex × age, $F_{(1108)} = 7.40$, $P < 0.01$. Fig. 2 illustrates the profiles of locomotor activity of all groups.

In the open field carried out with 45-day-old offspring, no significant differences in the number of squares crossed by both the control and F-treated groups were detected (Fig. 2A). In contrast, in 90-day-old offspring, post hoc comparisons showed that female and male offspring exposed to F exhibited a significant decrease in locomotor activity,

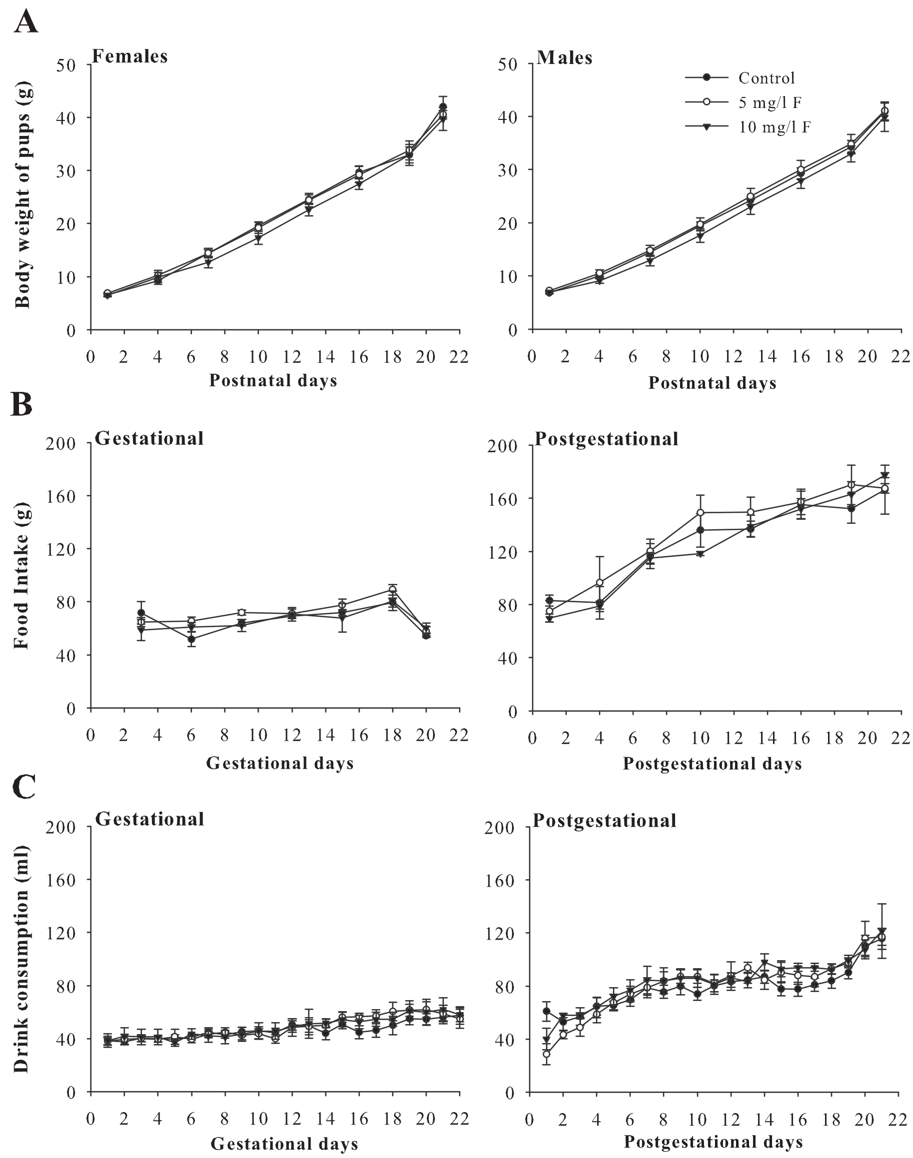

Fig. 1. Data of the mothers and their litters. A) Body weight of female (left) and male (right) pups on different postnatal days (1, 4, 7, 10, 13, 16, 19, and 21) in the control group and in the fluoride-exposed group (5 and 10 mg/L) groups. B and C) Food intake and drink consumption respectively during pregnancy (gestational days 3, 6, 9, 12, 15, 18, and 20) and lactation (postgestational days 1, 4, 7, 10, 13, 16, 19, and 21) of the different groups. Data represent the mean ± SEM of 10 mothers and their litters.

compared to the control group ($P < 0.001$ for male and female rats exposed to the two concentrations of F tested; Fig. 2B).

As to the other parameter of locomotor activity analysed, three-way ANOVA showed no significant differences in the number of rearings done by the control and F-treated groups at both ages during a 15 min period (Fig. 2).

When we compared the number of squares crossed and rearings performed every 5 min, the ANOVA for repeated measures showed significant differences intra-subjects in squares ($F_{(2216)} = 748.12$, $P < 0.001$) and rearings ($F_{(2216)} = 383.85$, $P < 0.001$). The number of squares crossed and rearings performed in each 15 min period was used to analyse habituation to the open field. All groups of offspring showed greater locomotor activity and a high number of rearings during the first 5 min period and declined in the second and third period ($P < 0.05$ for all groups with respect to the first period of 5 min). This

Table 2
Mean postnatal day on which control and F-treated groups displayed the criterion level of righting reflex, cliff aversion, and negative geotaxis.

		Righting reflex	Cliff aversion	Negative geotaxis
Control group	♀	4.2 ± 0.1	6.3 ± 0.1	10.9 ± 0.3
	♂	4.4 ± 0.1	6.3 ± 0.2	10.5 ± 0.2
5 mg/l F	♀	4.5 ± 0.1	6.7 ± 0.3	11.9 ± 0.5
	♂	4.2 ± 0.1	6.6 ± 0.3	11.4 ± 0.3
10 mg/l F	♀	4.2 ± 0.1	7.0 ± 0.3	10.6 ± 0.4
	♂	4.4 ± 0.2	6.5 ± 0.3	10.4 ± 0.2

Values are mean ± SEM. $n = 10$ females (♀) and 10 males (♂).

Table 3
Mean postnatal day on which both eyes were opened and on which both auditory canals were fully opened.

		Eyes	Ears
Control group	♀	13.7 ± 0.2	12.1 ± 0.1
	♂	13.9 ± 0.1	12.4 ± 0.1
5 mg/l F	♀	15.0 ± 0.1***	12.0 ± 0.1
	♂	14.8 ± 0.1***	12.1 ± 0.1
10 mg/l F	♀	14.5 ± 0.1**	12.5 ± 0.2
	♂	14.3 ± 0.1*	12.2 ± 0.1

Values are mean ± SEM. $n = 10$ females (♀) and 10 males (♂). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ with respect to the corresponding male/female control group.

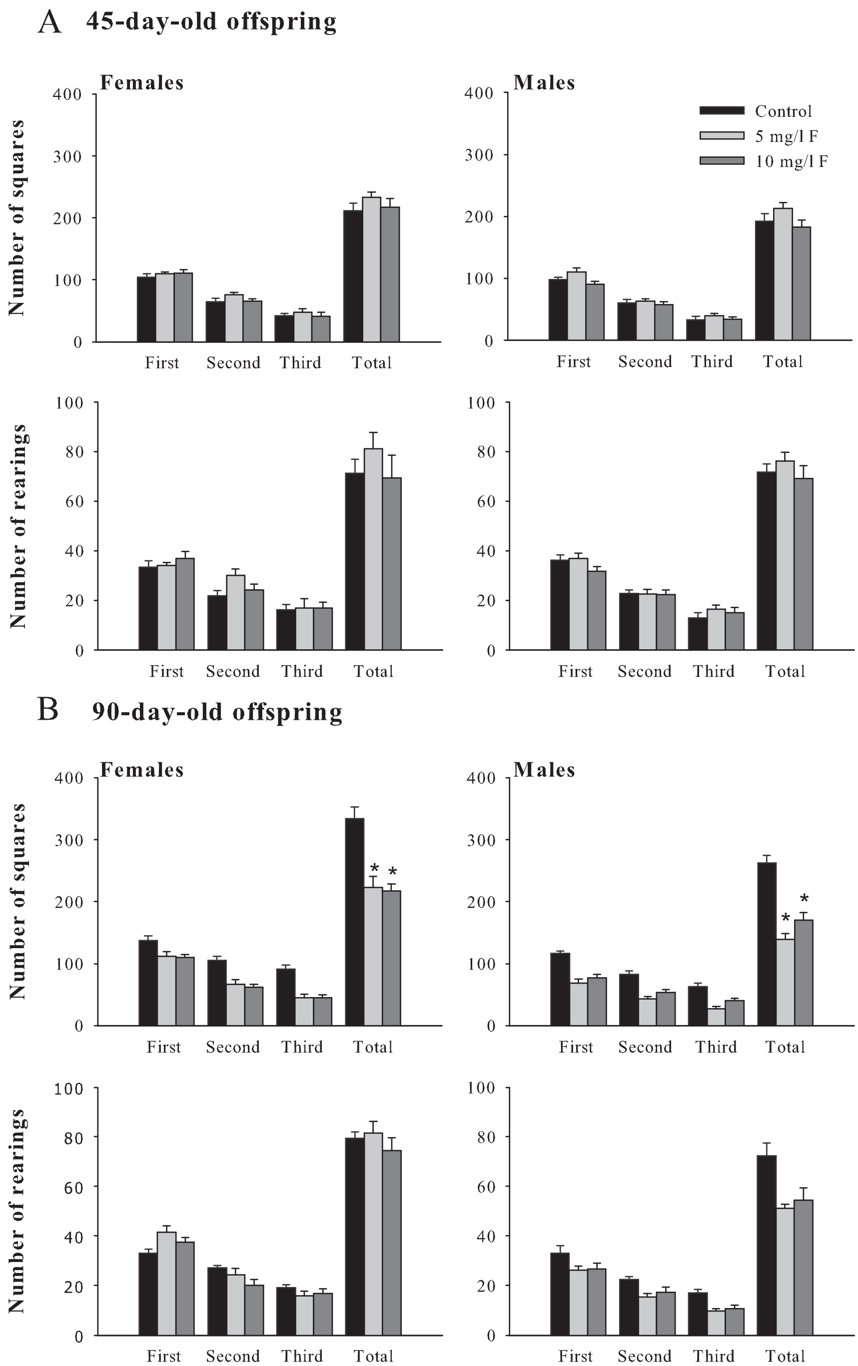

Fig. 2. Open field test in 45- and 90-day-old offspring. Number of squares crossed and rearings in each 5 min period and in the 15 min total test in the 45- (A) and 90-day-old (B) female and male offspring of the control and fluoride-exposed (5 and 10 mg/l) groups. Data represent the mean ± SEM of 10 females and 10 males per group. *$P < 0.001$ with respect to the control group.

gradual and significant decrease in their locomotor activity throughout the test session indicates that all animals had been habituated to the open field.

On the other hand, the emotionality parameters were analysed by three-way ANOVA, and significant differences were found in the number of groomings between sexes, $F_{(1108)} = 11.47$, $P < 0.001$, and ages, $F_{(1108)} = 4.07$, $P < 0.05$. Three-way ANOVA also revealed significant differences in the interaction between group × age, $F_{(2108)} = 12.77$, $P < 0.001$. In addition, post hoc comparisons showed that 90-day-old male offspring exposed to the two F concentrations tested ($P < 0.001$ and $P < 0.01$ for 5 and 10 mg/l F, respectively) and female exposed to 5 mg/l F ($P < 0.01$) exhibited a significant increase in the number of groomings compared to the control group (Table 4). As to the other parameter of emotionality analysed (number of faecal boluses), three-way ANOVA showed significant differences among groups, $F_{(2108)} = 11.87$, $P < 0.001$, and ages, $F_{(1108)} = 16.30$, $P < 0.001$. In 45-day-old offspring, post hoc comparisons showed that only the male offspring exposed to 5 mg/l F showed an increase in the number of faecal boluses compared to the control group ($P < 0.01$; Table 4).

Summing up, on postnatal day 45, the animals exposed to F during pregnancy and lactation, showed locomotor activity levels similar to those of controls. However, on postnatal day 90, the male and female offspring exposed to the two F concentrations tested, showed a significant decrease in locomotor activity. In addition, an increase registered in

Table 4
Number of groomings and faecal boluses as emotionality parameters.

		45-day-old offspring		90-day-old offspring	
		Groomings	Faecal boluses	Groomings	Faecal boluses
Control group	♀	5.10 ± 0.85	1.10 ± 0.55	2.80 ± 0.50	0.00 ± 0.00
	♂	7.90 ± 0.85	1.60 ± 0.75	2.75 ± 0.45	0.35 ± 0.20
5 mg/l F	♀	3.70 ± 0.50	2.90 ± 1.05	6.20 ± 1.15*	1.90 ± 1.20
	♂	5.60 ± 1.10	6.30 ± 0.90*	7.80 ± 1.20**	1.90 ± 0.90
10 mg/l F	♀	5.90 ± 1.05	1.80 ± 0.90	3.50 ± 0.55	0.50 ± 0.25
	♂	7.10 ± 0.90	2.20 ± 0.95	6.20 ± 0.75*	0.60 ± 0.30

Values are mean ± SEM. $n = 10$ females and 10 males. *$P < 0.01$, **$P < 0.001$ with respect to the corresponding male/female control group.

the number of groomings and faecal boluses largely of offspring exposed to the two F concentrations tested revealed higher emotionality.

3.4. Plus maze

The three-way ANOVA showed significant differences in the percentage of time spent in the open arms between groups, $F_{(2108)} = 23.17$, $P < 0.001$ and significant differences in the interaction between group × age, $F_{(2108)} = 3.73$, $P < 0.05$. In 45-day-old offspring, post hoc comparisons showed that only the female offspring exposed to the two F concentrations tested spent more time in the open arms compared to the control group ($P < 0.01$ and $P < 0.05$ for 5 and 10 mg/l F, respectively; Fig. 3A). On the other hand, in 90-day-old offspring, post hoc tests revealed that the female and male offspring exposed to 5 and 10 mg/l F during pregnancy and lactation, spent more time in the open arms than those of the control group ($P < 0.01$ for male and females at 5 mg/l F respectively, and $P < 0.001$ for both sexes at 10 mg/l F, respectively; Fig. 3B).

When the percentage of entries in the open-arm position was analysed, three-way ANOVA showed again significant differences in groups, $F_{(2108)} = 25.48$, $P < 0.001$, and ages $F_{(1108)} = 4.99$; $P < 0.05$; and in the interaction between group × age, $F_{(2108)} = 7.76$, $P < 0.001$. Post hoc comparisons showed that only the 90-day-old offspring exposed to the two F concentrations tested exhibited a significant increase in the percentage of entries in the open-arm position in both sexes compared to the control group ($P < 0.001$ for all comparisons; Fig. 3B). Three-way ANOVA showed no significant differences among group, age and sex, nor interactions among them when the total number of arm entries was analysed (data not shown).

Results derived from the plus maze test showed a lower index of anxiety in young females and all 90-day-old offspring exposed to 5 and 10 mg/l of F during pregnancy and lactation.

4. Discussion

Previous research has shown that fluorides interfere with the functions of the brain and other tissues. The present study was therefore planned to study the neurobehavioural effects of the exposure to low levels of F during pregnancy and lactation on female and male rat offspring.

It is known that the foetus is not well protected against xenobiotics that circulate in the maternal blood. This is because the placenta does not block the passage of several environmental toxicants from maternal circulation to foetal circulation [20]. More than 200 foreign chemicals have, in fact, been detected in the umbilical cord blood [21]. Further research has demonstrated that several environmental chemicals are transferred to the infant through human breast milk [20]. During foetal life and early infancy, the blood–brain barrier provides only partial protection against the entry of chemicals into the central nervous system [22]. Furthermore, the developing human brain is exceptionally sensitive to injury caused by toxic chemicals [23], and several developmental processes have been shown to be highly vulnerable to chemical toxicity. Recent research on well documented neurotoxicants has yielded important new insights into the neurodevelopmental consequences of early exposure to these industrial chemicals. A meta-analysis of 27 cross-sectional studies of children exposed to F through drinking water, mainly from China, suggests an average intellectual coefficient decrement of about seven points in children exposed to high F concentrations [24].

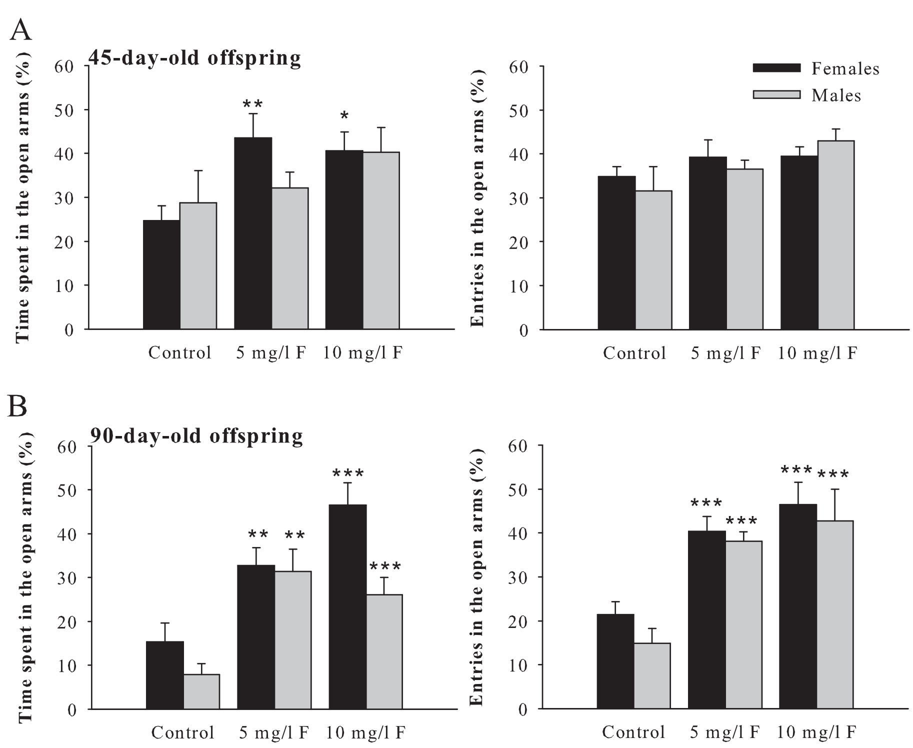

Fig. 3. Plus maze test in 45- and 90-day-old offspring. Percentage of time spent in the open arms and entries in the open arms measured in the plus maze over the 5 min test in 45- (A) and 90-day-old (B) female and male offspring. Data represent the mean ± SEM percentage of 10 animals per group per age. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ with respect to the control.

In the present research, it is shown that exposure to low levels of F during pregnancy and lactation does not affect maternal weight gain during pregnancy, gestational length, litter size, and body weight of pups on different postnatal days. Still, a significant delay in the development of eye opening was observed in the offspring whose mothers had been exposed to 5 and 10 mg/l F, thus suggesting an asynchrony of maturation processes during postnatal development. There is no literature, in fact, on the effects derived from exposure to F on sensorimotor reflexes nor on eye opening. Other chemical elements, such as aluminium and arsenic, have been observed to produce a significant delay in eye opening in offspring prenatally exposed to these compounds [25,26]. This is indicative of a delay in maturational development as a result of exposure to xenobiotics. The changes in their development and expression could represent a predictive factor for other behavioural modifications in adulthood [27].

In the present study, the decreased number of squares crossed during a 15 min observation period by 90-day-old rats in the open field test carried out was indicative of low locomotor activity in the female and male offspring exposed to 5 and 10 mg/l of F. In addition, an increase registered in the number of groomings and faecal boluses largely of offspring exposed to the two F concentrations tested revealed higher emotionality. No significant changes in locomotor activity nor in grooming were observed in the young offspring exposed to the two F concentrations tested. Previous research carried out on rats exposed to 500 mg/l NaF through drinking water during 60 days showed that locomotor activity was inhibited in adult rats [28]. This was associated with a locomotor behavioural deficit observed in workers with chronic exposure to high levels of industrial F [9]. On the other hand, it is known that the activation of mesolimbic dopamine pathways stimulates locomotor activity [29]. Other pathways, such as serotonergic, are involved in locomotion regulation [30]. F exposure during pregnancy and lactation may produce a delayed alteration in these neurotransmitters in 90-day-old male and female offspring exposed to the two F concentrations tested, and these alterations could be responsible for the hypoactivity observed in the experimental groups.

As to the results regarding anxiety, the present research shows that exposure to 5 and 10 mg/l F during pregnancy and lactation reduced anxiety levels in all female (45- and 90-day-old) and adult male offspring compared to those of the control group. This effect in the plus maze test was revealed in the elevated time that they spent in the open arms and in the high number of entries in the same arms. In addition, while the 45-day-old female offspring exposed to the two F concentrations tested were found to be less anxious with respect to the control group, no significant differences were observed in their male counterparts. This finding was not surprising as there is a general consensus according to which female rats are more sensitive to the toxic effects of chemicals than males [31,32]. On the other hand, Palanza [33] observed that in animal models of anxiety and depression the development of sexual dimorphisms in behaviour and cognitive function depends mainly on the action of gonadal hormones. In this work, also it was reported that gonadal hormones contribute to either the anatomic or functional characteristics of several neurotransmitter and neuromodulatory systems.

Several studies have confirmed that anxiety is regulated by gabaergic (GABA) and serotonergic (5-HT) systems. In line with this, while the increase in GABA activity produces a decrease in anxiety, the decrease in 5-HT neurotransmission leads to anxiolytic effects [34]. It was found that the content of hypothalamic serotonin increases during subacute fluorosis but decreases during chronic fluorosis [35,36]. Although no studies have been carried out to date on changes in GABA levels as a result of exposure to F, it has been observed that F readily penetrates into the brain and is accumulated in the brain tissues [37]. We therefore hypothesized that F produces changes in neurotransmission systems and that such changes are responsible for the effects observed in the offspring exposed to F during pregnancy and lactation.

Recent evidence indicates that high F concentrations (500 mg/l) produce neuronal destruction and synaptic injury by a mechanism that involves free radical production and lipid peroxidation. In this respect, previous research [38] has indicated that exposure of F in mice during pregnancy and lactation can induce functional changes, metabolic disorders, and histopathological alterations of some organs, and that these changes are due to F-induced oxidative stress of both mothers and their pups [38]. Also, a marked increase in oxidative stress, lipid peroxidation and a decrease in the activity of antioxidant enzyme were found in brain regions of rats chronically exposed to 100 mg/l F through drinking water [39]. Furthermore, chronic F exposure (100–200 mg/l) has been found to increase neuronal lipid peroxidation and to decrease the activity of antioxidant enzymes in 3 generations [10]. Further studies are therefore necessary to elucidate whether or not oxidative stress is responsible for the neurological changes herein reported.

Taken together, our findings lead us to conclude that exposure to the two F concentrations tested during pregnancy and lactation produces a delay in eye opening development in offspring as well as hypoactivity in adult offspring. It also yields a decrease in anxiety as shown by the plus maze test. All these changes indicate that F exposure during pregnancy and lactation, even at low concentrations, may alter parameters of the central nervous system functionality. Exposure to developmental neurotoxicants in early life is at present linked to specific clinical syndromes in children. The persistent decrements in intelligence documented in children, adolescents, and young adults exposed in early life to F may presage the development of neurodegenerative disease later in life. Thus, further studies will be crucial to elucidate the molecular mechanisms through which F exposure during gestation and lactation trigger neurobehavioral changes.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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