



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



Mariana Bartos^{a,*}, Fernanda Gumilar^a, Cristina Bras^a, Cristina E. Gallegos^a, Leda Giannuzzi^b, Liliana M. Cancela^c, Alejandra Minetti^a

^a Laboratorio de Toxicología, INBIOSUR-CONICET, Universidad Nacional del Sur, 8000 Bahía Blanca, Argentina

^b Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, 1900 La Plata, Argentina

^c Departamento de Farmacología, IFEC-CONICET, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina

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.

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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

* Corresponding author at: Laboratorio de Toxicología, INBIOSUR-CONICET, Universidad Nacional del Sur, San Juan 670, 8000 Bahía Blanca, Argentina.

E-mail address: mbartos@criba.edu.ar (M. Bartos).

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 ($22^{\circ} \pm 1^{\circ}\text{C}$) 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 $50 \times 50 \times 60$ 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.

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