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BEHAVIORAL TERATOLOGY IN RATS EXPOSED TO FLUORIDE

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[SUMMARY: A study was conducted of behavioral differences in Wistar rat pups born of dams drinking water containing 1.0, 5.0, and 25 mg F/L from the day of conception. A control group drank tap water containing 0.6 mg F/L. Among 18 litters in the 25 mg/L group, four of the dams failed to produce enough milk for their pups, causing them to starve. Although no differences or abnormalities in physical development were observed, differences in motor coordination, auditory reaction, pain sensitivity, and other cognitive responses, some statistically significant, varying with time and F exposure, were noted, especially among the pups in the 25 mg/L group. Brain slices in the 25 mg/L group also showed a significantly lower average cerebral cortex thickness than in the control group (10.97 μm vs. 11.70 μm).]

[Keywords: Auditory response; Behavioral teratology; Locomotion development; Offspring rats and fluoride.]

INTRODUCTION

Fluoride is reported to pass through the placental barrier and into the body of a growing fetus, penetrating the blood-brain barrier and accumulating in brain tissue.^{1,2} The present study used methods of behavioral teratology to investigate the behavioral development of the offspring of rats exposed to low doses of fluoride during pregnancy.

MATERIALS AND METHODS

1. Selection and handling of the laboratory animals:

Adult Wistar rats, each weighing approximately 250 g, were provided by the Laboratory Animal Center of the Chinese Medical Science Institute. They were initially kept in cages with a 2-to-1 female:male ratio. The date that a vaginal smear test was discovered to contain sperm was counted as day 0 of the pregnancy. Starting from that day, the rat dams were allowed free access to tap water that had been first boiled and then cooled, with a final fluoride concentration of 1 mg/L, 5 mg/L, or 25 mg/L, depending on the group to which the dams belonged. The control group was given free access to the same tap water containing 0.6 mg F/L with no additional fluoride. Another group was not given fluoridated water during pregnancy; however, drinking water with a 25 mg F/L was supplied beginning on the day the offspring were born. The size of each litter was controlled to 6 to 8 in size, and during the nursing period the dams continued to drink water with the same fluoride concentration. The offspring rats also drank the same water after weaning.

2. Behavioral teratology testing:

Behavioral teratology testing of the offspring rats was conducted according to procedures outlined in the literature.^{3,4}

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RESULTS

1. Dams:

There were no significant differences in the weight of the dams or the size of their litters. However, for the 18 litters born to rats drinking 25 mg/L fluoridated water, four of the dams did not produce milk, and two others were low in their production of milk; the rats in these litters eventually starved. This phenomenon did not appear in any of the other groups, and it may indicate that fluoride might have an inhibiting effect on milk production.

2. Offspring rat pups:

(i) *Standard physical development:* With regard to the weight of the rats in the various developmental stages after birth, there was no significant difference between the various groups of exposed rats as compared to the control group. Characteristic features of physiological development such as the time of pinna detachment, incisor eruption, and eye opening showed no significant differences between exposed rats and the control rats.

(ii) *Reflex and sensory development:* Surface righting, cliff avoidance, and negative taxis testing each showed no differences between the various groups of exposed rats and the control. On day 7, the time required for the pivoting task was noticeably longer for the 25 mg F/L group ($p < 0.05$); however, there was no significant difference between any of the groups by day 9. The freezing response to auditory stimuli for the 25 mg/L group was significantly delayed ($p < 0.01$), and, when the rats were subjected to hot plate testing on day 45, there were significant differences in the pain response latency period ($p < 0.01$). In the control and 1 mg/L groups, male and female rats showed significant differences in pain response, but in the 5 mg/L, 25 mgF/L, and post-birth 25 mg/L groups these sex differences were no longer significant (Table 1).

Table 1. Pain response latency period in offspring at day 45, by age (mean±SD)

Sex	n	Control	1 mg F/L group	5 mg F/L group	25mg F/L group	Post-birth 25mg F/L group
Male	20	2.43±0.39*	2.00±0.52*	1.94±0.29	3.01±0.46 [†]	2.56±0.56
Female	20	2.14±0.34	1.67±0.37	1.80±0.34	2.82±0.53 [†]	2.40±0.59

* $p < 0.05$ comparison between the sexes; [†] $p < 0.01$ as compared to the control.

(iii) *Motor coordination development:* The results of swimming testing conducted between 9 and 19 days show no significant differences between groups for the offspring in the same development phase with regard to swimming method, swimming angle, or limb usage. On day 20, 24, and 28, a significant increase in leg extension by the 25 mg/L group as compared to the other groups was observed ($p < 0.05$, $p < 0.01$). In developmental phases I and II, there were no significant differences in locomotion; in phase III, however, the 5 mg/L group showed significantly delayed response ($p < 0.01$). No significant differences were observed in forelimb suspension time. In the slanted surface experiment, the number of animals in the post-birth 25 mg/L group that tested negative was significantly higher than the other groups, $p < 0.01$. In the open field test, the defecations, urinations, circlings, and droppings all showed no significant differences. The latency period for the post-birth 25 mg/L showed significant lengthening ($p < 0.01$).

Each of the fluoride-dosed groups showed drops in the frequency of face scrubbing ($p < 0.05$ or $p < 0.01$). The 5 mg/L and 25 mg/L groups each tended toward fewer rearings, and for the post-birth 25 mg/L group the differences were significant as compared to the control ($p < 0.01$), as the total number of squares traveled (Table 2).

Table 2. Open observation experiment results of the offspring at day 30

Group	Litters	n	Latency Period (s)	Face Scrubbings (No.)	Rearings (No.)	Squares Traveled (No.)
Control	11	40	4.36±2.7	3.9±3.3	33.5±13.2	141.5±29.5
1 mg/L	10	40	3.93±2.3	2.3±2.1*	39.8±13.6	145.5±29.3
5 mg/L	10	40	6.22±5.5	2.0±1.7*	24.9±15.2	114.3±26.5*
25 mg/L	11	40	5.05±3.1	2.0±1.4*	28.0±12.2	129.9±30.0
Post-birth 25 mg/L	9	40	7.79±7.3*	2.8±2.8*	18.8±10.4*	105.5±34.0*

* $p < 0.01$ as compared to the control.

(iv). *Cognitive function:* The results of water maze testing showed that the pups exposed to fluoride during the fetal development phase make more mistakes before discovering the correct path, and in the 25 mg/L group the increase was significant ($p < 0.05$). The time it took the 25 mg/L group and the post-birth 25 mg/L group to find the hidden platform was significantly lengthened, indicating that higher doses of fluoride cause definite learning deficits in exposed fetuses after birth. There were no significant differences between the sexes in their performance in this test (Table 3).

Table 3. The effects of fluoride on locomotion and water maze performance

Group	Litters	Locomotion (d)			Water maze (45-day-old rats)	
		Phase I	Phase II	Phase III	Training runs	Time to platform (s)
Control	11	6.1±1.2	10.1±1.2	13.6±0.9	4.3±1.3	7.09±2.18
1 mg F/L	10	6.4±0.7	9.5±0.5	13.5±0.8	4.0±1.6	8.28±2.71
5 mg F/L	10	7.9±0.9	10.1±1.1	14.9±1.1*	4.8±1.2	8.63±2.98
25 mg F/L	11	7.4±1.4	9.8±0.9	13.9±0.5	5.2±1.3*	9.54±4.16*
Post-birth 25 mg F/L	9	6.0±1.8	9.8±1.0	13.9±0.3	4.9±1.8	10.19±4.70†

* $p < 0.05$ compared to control; † $p < 0.01$ compared to control.

3. Brain slice pathology:

At day 21, 70, and 90, brain slices of the offspring were examined for pathological changes, and on day 21 the thickness of the cerebral cortex of the control and 25 mg/L groups were measured. The average control group pup's cerebral cortex was 11.70 μm thick versus 10.97 μm for the 25 mg/L group; the difference between the two is significant, indicating that fluoride can slow brain cell growth. The examination of brain slices showed focal neuronophagia in all of the 25 mg/L offspring at day 70 and 90; there were no significant pathological changes in the brain tissues of the other groups.

DISCUSSION

At present, numerous experiments have already demonstrated that developmental and behavior changes can occur in an organism exposed to doses less than that which would cause obvious structural irregularities. Using methods of behavioral teratology to evaluate the safety of chemical substances has already received significant attention. Due to the complexity as well as the highly adaptive nature of the central nervous system, a single behavioral test or a small group of

tests cannot properly reflect the effects of a particular factor on general behavior; a full test battery is required along with longitudinal study tracking changes over time.⁵ In the present experiment, after the rat dams were exposed to fluoride through their contaminated drinking water, there were no significant differences in the weight of offspring and size of litter; however, the proportion of dams drinking 25 mg F/L water that were low or completely lacking in milk rose significantly, indicating that fluoride has an inhibiting effect on milk production. Song Keqin et al.⁶ found that the prolactin in the pituitary glands of chronically fluoride poisoned rats exhibited changes in cell ultrastructure, and they theorize that fluoride inhibits the release of prolactin and perhaps even has a direct, toxic effect on prolactin cells. The results of our experiment support that conclusion. Based on the relevant indices, there was no significant delay in physical development in the offspring, suggesting that fluoride does not affect physical development at the doses used in this experiment.

In the literature there are reports that fluoride affects auditory function; the results of tests conducted on endemic fluoride-poisoned patients show that the damage to the auditory system caused by fluoride leads to a kind of nerve deafness to high frequency sounds, and the authors believe that fluoride's toxic effects on the eighth cranial nerve is the mechanism by which this occurs.⁷ Here the freeze response to auditory stimuli in the pups showed significant delay, indicating that relatively high doses of fluoride can negatively influence the development of auditory nerves. Guan Zhizhong et al.^[8] report that the offspring of rats exposed to fluoride have retarded cerebral development and exhibit changes in neural cell ultrastructure. The results of the present experiment suggest that the effects of high doses of fluoride on the behavior development of the offspring are visible primarily as slight delays in response times, particularly in regard to motor and coordination function as well as muscle strength. The measurement of the thickness of the cerebral cortex of offspring on day 21 revealed that the 25 mg/L group had a significantly thinner cerebral cortex as compared to the control. This histological analysis indicates that fluoride slows the growth of brain cells

In our study, two groups of offspring rats were exposed to high dosages of fluoride: one whose exposure began during fetal development, and the other whose first exposure occurred after birth, during nursing. The results indicate there were no significant differences between the two groups with respect to early development indicators. Locomotion and coordination functions, however, showed greater developmental delay in the rat pups exposed to fluoride at nursing as compared to those exposed to the same dose during the fetal stage. This difference could be due to the development of a tolerance to fluoride in the pups in the latter group. Reports in the literature show that long-term, exposure with slow increases in the fluoride concentration in cell cultures will cause cells to develop a tolerance to otherwise fatal doses of fluoride, even to the point where the fluoride does not measurably affect cell growth. The mechanism at work could be an adaptation of the cell membrane, blocking fluoride from entering the cell as well as actively removing fluoride already inside, and thus maintaining a low intracellular fluoride concentration.⁹

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