EFFECTS OF FLUORIDE AND LEAD ON LOCOMOTOR BEHAVIOR AND EXPRESSION OF NISSL BODY IN BRAIN OF ADULT RATS

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SUMMARY: By means of an activity chamber and a Y-maze, spontaneous and conditioned-response behaviors, respectively, of adult Wistar albino rats were determined after administration of sodium fluoride (150 mg/L) and/or lead acetate (300 mg/L) in their drinking water for 30 days. The dental status and the expression of Nissl body in the brain of the rats were also tested. Results showed that fluoride (F) and/or lead (Pb) altered the frequency and duration of spontaneous activities and adversely affect conditioned-response behaviors. In addition, the expressions of Nissl body were significantly decreased in the treatment groups compared with the control group, whereas no changes were observed in the dental status among different groups. These findings indicate that F and/or Pb can influence spontaneous behaviors and lower the learning ability of rats before the appearance of dental lesions.

Keywords: Fluoride and brain function; Lead; Nissl body; Rat brain; Spontaneous behavior; Y-maze test.

INTRODUCTION

Accumulated evidence indicates that prolonged ingestion of fluoride (F) produces deleterious effects on skeletal,[1,2] dental,[3-6] and soft tissues including brain,[7-10] thyroid,[11-14] and testis.[15-18] F exposure often also occurs concurrently with other environmental contaminants like lead (Pb),[19-21] a persistent and pervasive industrial environmental contaminant throughout the world. Although the toxic effects of F or Pb alone have been studied extensively, their combined effects on the central nervous system (CNS) are of special concern. Among neurotoxic investigations, neurobehavioral tests are usually applied to detect the neurotoxic effects of neurotoxins. Spontaneous activity, a motivated behavior, is considered to be the most common “predictor” of CNS dysfunction.[25] In addition, conditioned-response tests can reflect the specific responses of animals to the presentation of stimuli.

In the present study, an activity chamber and a Y-maze were used to measure the spontaneous activity and conditioned-response behavior of adult rats in response to exposure to F and Pb.

MATERIALS AND METHODS

Establishment of animal model: Forty adult Wistar albino rats, with a mean body weight of 180±5.3 g, were obtained from the experimental animal center of Shanxi Medical University and kept in plastic cages in our laboratory and maintained on their standard diets. After one week, the rats were divided randomly into four groups of ten animals each (male: female=1:1). One group as control received double distilled water, the other three groups were treated with sodium fluoride (150 mg NaF/L: HiF group), lead acetate (300 mg/L: HiPb group), and sodium

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fluoride (150 mg NaF/L) plus lead acetate (300 mg/L: HiF+HiPb group) in their drinking water. Animals had free access to food and water for 30 days and were maintained on their normal diets under standard temperature (22–25°C), 12/12-h light/dark cycle, ventilation, and hygienic conditions. The study design was approved by the Institutional Animal Care and Use Committee of China.

Spontaneous activity test: At the end of the foregoing 30-day conditions, the spontaneous ability of the rats was observed in each group in an activity chamber similar to that reported in prior study of Mullenix et al.[22] with small differences. The chamber consists of a clear glass box with two video cameras fixed at the top and one camera on one side of the chamber to monitor the spontaneous behavior of each rat over a 15-min period. The video signals were transferred to a video-splitter device to record at the rate of one frame per second. Behavioral initiations (BI) and behavioral total time (BTT) were applied to measure frequency and duration of the following five body positions for each rat: sitting, standing on four feet, standing on two feet, head turning, and walking.

Y-maze test: A Y-maze consisting of three equal-sized arms with a 120° angle between each of the two arms, was used to determinate the learning ability of rat after the spontaneous activity test. The principle of this apparatus is the same as that described by Zhang et al.[23] During the training days, the test for each rat was run 20 times, and then another 20 runs were carried out at the same time next day. The tests were continued until the rats had mastered the test. Eighteen correct reactions in 20 consecutive runs (90% success rate) were considered to be the learning standard. The error number (EN) of each rat was recorded before it mastered the test. Two additional measurements for the learning test were the number of days required for the rat to meet the 90% learning standard and the total reaction time (TRT) for summing the reaction time required for each rat per test day. All training tests were carried out between 21:00 and 23:00, in quiet and dim conditions and were completed in less than one week.

Tissue preparation: When the behavior tests were completed, the rats were anesthetized with 20% urethane (ethyl carbamate, $NH_2COOC_2H_5$) solution. The brains were quickly removed and fixed, dehydrated, and embedded by paraffin for Nissl stain. The teeth were collected and then stored at –80ºC for radiological examination by X-ray.

Nissl stain: The embedded brain samples were cut into serial coronal sections with the thickness of 5 μm. The section was performed with routine Nissl staining based on toluidine blue technique. The images of sections were captured by an upright microscope (model: BX51, OLYMPUS of Japan), and the gray value of the stained Nissl bodies was determined by Image-Pro® Plus Version 5.1 micrograph analysis software (made in Media Cybernetics Inc. of America).

Statistical analyses: EN and learning days in the behavioral data were analyzed by nonparametric test followed by Chi-square test. The other data were evaluated by one-way ANOVA (analysis of variance) followed by the LSD (least significant difference) test with $p<0.05$ being considered significant. The results are expressed as mean ± SEM.

RESULTS

Spontaneous activity test: Table 1 shows that HiF and/or HiPb had an obvious impact on the frequency and duration of sitting, standing on four feet, standing on two feet, walking, and head turning. Behavior initiation (BI) of sitting in the treatment groups was significantly suppressed, while the behavioral total time (BTT) of this action was prolonged. As for standing on four feet and on two feet, although there were no significant differences in BI, the BTT were markedly changed in three treatment groups compared with the control group. F and/or Pb obviously shortened the BTT of walking and head turning, except for the BTT of head turning in the HiF group, which was increased significantly.

Table 1. Behavioral initiations (BI) and behavioral total time (BTT) in seconds of spontaneous behavior in rats exposed to F and/or Pb (n=10; mean±SEM)

Behavior		Control	High fluoride (HiF)	High lead (HiL)	High fluoride and high lead (HiF+HiL)
Sitting	BI	23.90±2.33	14.75±1.26†	11.17±2.76†	11.36±1.12†
	BTT	87.90±4.01	100.50±12.92†	102.17±8.87†	96.18±7.19†
Standing on four feet	BI	50.70±18.35	48.25±20.95	62.50±24.77	57.82±19.12
	BTT	261.9±5.82	152.00±15.10†	372.58±24.83†	335.00±13.91†
Standing on two feet	BI	47.00±21.15	76.50±22.78	63.00±25.00	53.73±30.46
	BTT	253.90±10.04	442.50±8.81†	178.83±10.61†	181.27±5.92†
Walking	BI	43.50±11.16	58.00±17.10*	64.5±25.31	58.36±22.98
	BTT	251.2±14.81	151.50±12.97†	206.58±15.23†	234.09±10.04
Head turning	BI	35.50±5.14	52.00±9.80*	36.83±4.90	26.18±4.56*
	BTT	45.10±4.25	53.50±7.55*	39.84±4.20*	26.73±4.72†

*p<0.01; †p<0.01 compared with the control group.

Y-maze test: As seen in Figure 1, rats in the three treatment groups spent more days in meeting the learning standard of the Y-maze test. From Figure 2 we can see that F and/or Pb significantly increased the error number (EN) of rats during the days of training. Compared with the controls, the TRT was significantly changed only in the HiF+HiPb group on the 1st day of training, and in the HiF group on the 2nd and 3rd day of training (Figure 3).

Radiological Features: Radiographs of the teeth in Figure 4 show the tooth status of adult rats exposed for 30 days to HiF, HiPb, and HiF+HiPb. From these radiographs it can been seen that the appearance of the teeth was smooth with no changes in tooth density, thus showing that dental fluorosis was not evident in the treatment groups compared with the control.

Nissl body staining: As seen in Table 2, the expression of Nissl body in the treated rats was decreased. The gray values of Nissl staining in the HiF ($p<0.01$), HiPb ($p<0.05$), and HiF+HiPb groups ($p<0.01$) were significantly lower than that in the control group.

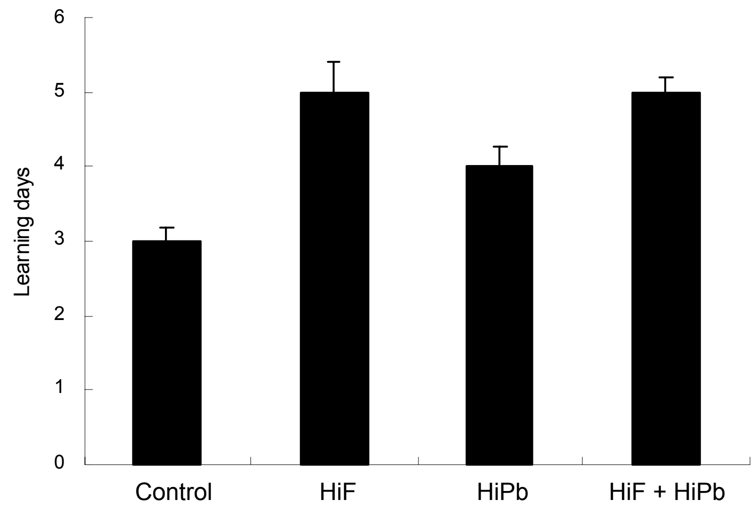

Figure 1. Learning days during Y-maze test of adult rats in control, HiF, HiPb, and HiF+HiPb groups.

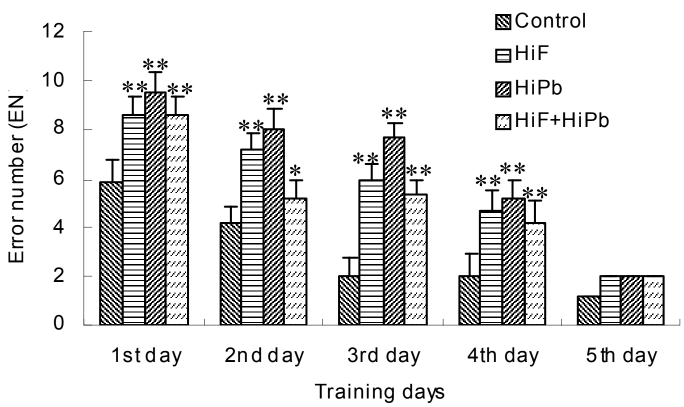

Figure 2. Error number (EN) during Y-maze test of adult rats in control, HiF, HiPb, and HiF+HiPb groups.

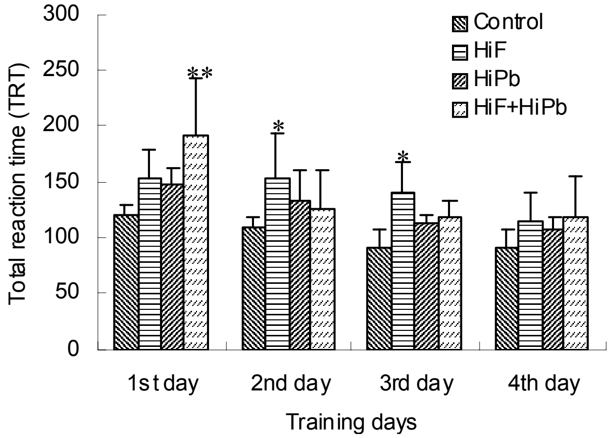

Figure 3. Total reaction time (TRT) during Y-maze test of adult rats in control, HiF, HiPb, and HiF+HiPb groups.

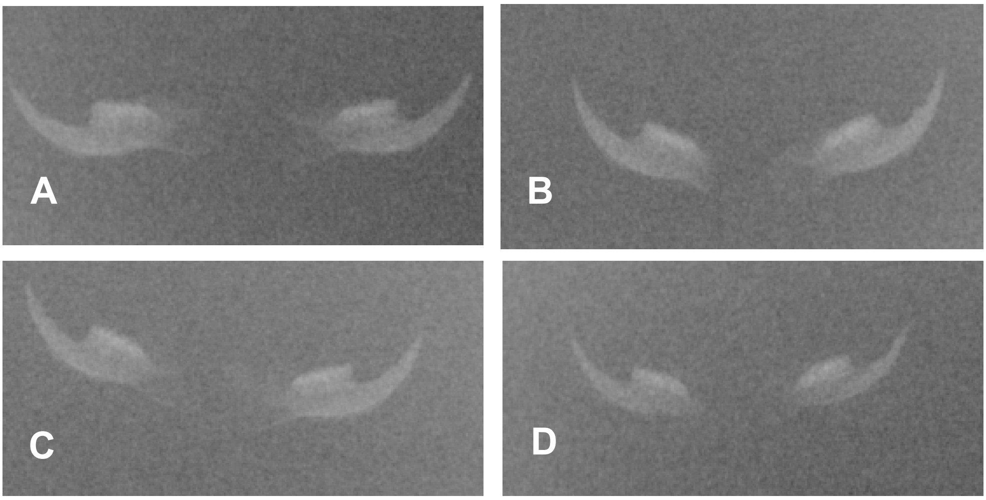

Figure 4. Radiographs showing the tooth status of adult rats exposed to HiF, HiPb, and HiF+HiPb for 30 days. Compared with the control group (A), HiF group (B), HiPb group (C), and HiF+HiPb group (D) exhibited smooth appearance and no changes in tooth density.

Table 2. Expression of Nissl body measured as gray value in brain of adult rats exposed to HiF, HiPb, and HiF+HiPb for 30 days (mean±SEM, n = 10)

	Control group	HiF group	HiPb group	HiF+HiPb group
Gray value	0.4237±0.1112	0.2713±0.0874†	0.3154±0.0325*	0.2486±0.0891†

$^{*}p<0.05$, $^{\dagger}p<0.01$ compared with the control group.

DISCUSSION

Effects of F and/or Pb on behavior: Since the behavior of both humans and animals is the product of what occurs in the nervous system, behavioral analysis is an essential assay of neural function.[24] The present study of spontaneous behavior in rats included monitoring of major body positions and movements like sitting, standing, and walking, as well as modified activities such as scratching and smelling, as examples of motivated behavior.[25] We found an inhibition of some of these body positions and activities were impaired by HiF and/or HiPb exposure. The results in the HiF group were consistent with animal data reported earlier.[22,26,27] Among these studies, however, Ekambaram also found lesions in hard tissues, which could in part lead to neurobehavioral deficits. In contrast, in the present study, the radiological features of the rat teeth determined by X ray showed no difference between control and treated groups. Thus, we postulate that changes in behavior occurred before the dental lesion, indicating that damage in hard tissue is not the direct factor for nervous system dysfunction caused by F.

From results of the Y-maze test, we can see a significant decrease in learning ability of animals in the HiF+HiPb group. It is well known that Pb can weaken the CNS by simulating the biochemical function of calcium.[28] In addition, the impact on calcium function is also one of the toxic mechanisms of F.[29] Hence combined effects of F and Pb may aggravate neural defects.

Effects of F and/or Pb on Nissl body substance: It is well documented that F exposure can induce the loss of neuronal cell bodies and damage synaptic structures in different regions of brain[30,31] as well as cause inhibition of enzyme activity[8,32] and a decrease in expression of membrane proteins.[10] Nissl body is an important component in neuronal cytoplasm, which exists in neurons except in the axon hillock. The chemical composition of Nissl body includes ribonic acid and proteins (ribosomes), and it is the main location for neural protein synthesis. Nissl body is very sensitive to pathological stimulus and is therefore usually considered a good index of neurocyte injury. In the present study, the expression of Nissl body was significantly decreased in treated groups, which was corroborated by the occurrence of cognitive dysfunctions in the treatment animals.

In summary, the results of this work demonstrated that F and Pb adversely influenced the locomotor behavior of rats before the occurrence of dental lesions.

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REFERENCES

1 Botha CJ, Naudé TW, Minnaar PP, Van-Amstel SR, Jansevan-Rensberg SD. Two out breaks of fluorosis in cattle and sheep. J S Afr Vet Assoc 1993;64(4):165-8.
2 Wang JM, Cheng XF, Zhou BH, Yan XY, Guo YX, Wang JD. Effects of protein and calcium on bone metabolism and biomechanical indexes in malnutrition-fed rabbits exposed to high fluoride. Fluoride 2008;40(1):46–63.
3 Wang JD, Guo YH, Liang ZX, Hao JH. Amino acid composition and histopathology of goat teeth in an industrial fluoride polluted area. Fluoride 2003;36(3):177-84.
4 Wang JD, Hong JH, Li JP, Guo YH, Zhang JF, Hao JH. Effect of high fluoride and low protein on tooth matrix development in goats. Fluoride 2002;35(1):51-5.
5 He Y, Niu RY, Wang JM, Liu HT, Wang JD. Effects of protein versus calcium supplementation on bone metabolism and development in fluoride-exposed offspring rats fed protein- and calcium-deficient diets. Fluoride 2008;41(3):192-8.
6 Hicks MJ, Flaitz CM. Enamel caries formation and lesion progression with a fluoride dentifrice and a calcium-phosphate containing fluoride dentifrice: a polarized light microscopic study. ASDC J Dent Child 2000; 67(1): 21-8.
7 Ge YM, Ning HM, Wang SL, Wang JD. Effects of high fluoride and low iodine of brain histopathology in offspring rats. Fluoride 2005;38(2):127-32.
8 Bhatnagar M, Rao P, Saxena A, Bhatnagar R, Meena P, Barbar S, et al. Biochemical changes in brain and other tissues of young adult female mice from fluoride in their drinking water. Fluoride 2006;39(4):280-4.
9 Bhatnagar M, Rao P, Shukla S, Jain S. Neurotoxicity of fluoride: evidence of neurodegeneration in hippocampus of female mice. Ind J Exp Biol 2002;40(5):546-54.
10 Niu RY, Sun ZL, Cheng ZT, Liu HT, Chen HC, Wang JD. Effects of fluoride and lead on N-methyl-D-aspartate receptor 1 expression in the hippocampus of offspring rat pups. Fluoride 2008;41(2):101–10.
11 Susheela AK, Bhatnagar M, Vig K, Mondal NK. Excess fluoride ingestion and thyroid hormone derangements in children living in Delhi, India. Fluoride 2005;38(2):98-108.

12 Zhou BH, Wang HW, Wang JM, Zhang JH, Yan XY, Wang JD. Effects of malnutrition and supplemented nutrition on nonspecific immune function changes induced by fluoride in rabbits. Fluoride 2007;40(3):169-77.
13 Ge YM, Ning HM, Wang SL, Wang JD. DNA damage in thyroid gland cells of rats exposed to long-term intake of high fluoride and low iodine. Fluoride 2005;38(4):318-23.
14 Bouaziz H, Soussia L, Guermazi F, Zeghal N. Fluoride-induced thyroid proliferative changes and their reversal in female mice and their pups. Fluoride 2005;38(3):185-92.
15 Liu HT, Niu RY, Wang JM, He Y, Wang JD. Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats. Fluoride 2008; 41 (3):184-91.
16 Zhang JH, Liang C, Ma JJ, Zhou BH, Wang JD. Changes in testis protein and metabolic enzyme activities in rats induced by sodium fluoride and sulfur dioxide. Fluoride 2006;39(3):179-84.
17 Zhang JH, Liang C, Ma JJ, Zhou BH, Wang JD. Effects of sodium fluoride and sulfur dioxide on oxidative stress and antioxidant defenses in rat testes. Fluoride 2006;39(3):185-90.
18 Wan SX, Zhang JH, Wang JD. Fluoride-induced changes in the expression of epidermal growth factor and its receptor in testicular tissues of young male rats. Fluoride 2006;39(2):121-5.
19 Maas RP, Patch SC, Christian AM, Coplan MJ. Effects of fluoridation and disinfection agent combinations on lead leaching from leaded-brass parts. Neurotoxicology 2007; 28(5):1023-31.
20 Masters RD, Coplan MJ, Hone BT, Dykes JE. Association of silicofluoride treated water with elevated blood lead. Neurotoxicology 2000;21(6):1091-100.
21 Coplan MJ. Patch SC, Masters RD, Bachman MS. Confirmation of and explanations for elevated blood lead and other disorders in children exposed to water disinfection and fluoridation chemicals. Neurotoxicology 2007;28(5):1032-42.
22 Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. Neurotoxicity of sodium fluoride in rats. Neurotoxicol Teratol 1995;17(2):169-77.
23 Zhang ZG, Xu XL, Shen XY, Xu XH. Effect of fluoride exposure on synaptic structure of brain areas related to learning-memory in mice. Journal of Hygiene Research, 1999;28(4):210-2.
24 Whishaw IQ, Haun F, Kolb B. Analysis of Behavior in Laboratory Rodents. In: Windhorst U, Johansson H, editors. Modern techniques in neuroscience research (with CD-ROM for Windows and Macintosh, Springer Lab. Manuals). Secaucus, New Jersey, USA: Springer Verlag; 1999. Chapter 44:1243-76. p.1243-4.
25 Mullenix PJ, Kernan WJ. Extension of the analysis of the time structure of behavioral acts. Intern J Neuroscience 1989;44(3-4):251-62.
26 Ekambaram P, Paul V. Calcium preventing locomotor behavioral and dental toxicities of fluoride by decreasing serum fluoride level in rats. Environ Toxicol Pharmacol 2001;9(4):141-6.
27 Paul V, Ekambaram P, Jayakumar AR. Effects of sodium fluoride on locomotor behavior and a few biochemical parameters in rats. Environ Toxicol Pharmacol 1998;6(3):187-91.
28 Choi DW. Calcium: still center-stage in hypoxic-ischemic neuronal death. Trends Neurosci 1995,18(2):58-60.
29 Hua k, Zhao H, Huang M, Li GS. Effects of fluoride on ionized calcium and calcium channel in osteoblast-like cell in vitro. Chin J Appl Phys. 2003;19(2):179-81.
30 Shivarajashankara YM, Shivashankara AR, Gopalakrishna B, Muddanna RS, Hanumanth RS. Histological changes in the brain of young fluoride-intoxicated rats. Fluoride 2002;35(1):12-21.
31 Shashi A. Histopathological investigation of fluoride-induced neurotoxicity in rabbits. Fluoride 2003;36(2):95-105.
32 Wu CX, Gu XL, Ge YM, Zhang JH, Wang JD. Effects of high fluoride and arsenic on brain biochemical indexes and learning-memory in rats. Fluoride 2006;39(4): 274-9.

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