

[Translated by Julian Brooke and published with the concurrence of the
Chinese Journal of Endemiology 2000;19(4):262-3.]

EFFECTS OF HIGH FLUORIDE DRINKING WATER ON THE CEREBRAL FUNCTION OF MICE

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SUMMARY: *Objective:* To study the effects after six months of exposure of mice to increasing concentrations of fluoride in drinking water (tap water, 10, 50, and 100 mg F/L) on their cerebral functions. *Methods:* Learning and memory abilities of high-fluoride exposed and control groups of mice were measured by a behavior-toxicological test (Shuttle-box Test), and the cholinesterase (ChE) activity in brain tissue homogenates of the mice was determined. *Results:* Learning and memory abilities of the fluoride-exposed groups were significantly lower than those of the control group, and the brain ChE activities of the fluoride-exposed groups were significantly higher. *Conclusions:* Elevated fluoride concentration in drinking water can decrease the cerebral functions of mice. Fluoride is a neurotoxicant.

[Keywords: Avoidance learning test; Behavior-toxicological test; Cerebral function; Cholinesterase; Electric shock; Mouse learning test; Fluoride as a neurotoxicant.]

INTRODUCTION

In recent years, with the expansion of studies on the skeletal effects of fluoride poisoning, research on non-skeletal effects, particularly damage to the nervous system, has received renewed attention. In order to investigate the effects of elevated levels of fluoride drinking water on the brain function of mice, we carried out a behavioral toxicology experiment on mice that had been drinking fluoride-containing water for six months; we also tested the levels of cholinesterase (ChE) in their brain tissue. The results give scientific credence to undertaking the prevention and control of non-skeletal damage resulting from drinking water-induced endemic fluoride poisoning.

MATERIALS AND METHODS

Grouping and handling of the animals: For the study, 60-day-old Kunming mice, each weighing 15 to 18 grams, were selected. Prior to the experiment, the mice were subjected to an initial selection process, with mice that showed behavioral deficits disqualified from the study. Then four groups were formed at random, each group including 12 mice with equal numbers of males and females.

One group was selected as the control group and given plain tap water to drink. The other three groups were designated as high fluoride group I, high F group II, and high F group III; they were given tap water containing 100, 50, and 10 mg F/L, respectively. All the rats were allowed unlimited access to food and water. After six months, the toxicology experiment was conducted, and samples of brain tissue were taken to test the cholinesterase activity levels.

Behavioral toxicology experiment: A shuttle-box was used to test the avoidance learning ability of the mice. Before the first run, each mouse was placed in the box and allowed to move around freely for 5–10 min to familiarize itself with the

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environment. Then the mouse was exposed to a conditional stimulus (a light) for 3 seconds in whichever room it happened to be in. If the mouse did not run to the adjoining room within the time limit, an unconditional stimulus was applied (an electric shock) concurrently with the existing stimulus and increased until the mouse ran into the next room, at which point both stimuli ceased. This was repeated 30 seconds later. If the mouse ran into the adjoining room while exposed to the conditional stimulus, then that was counted as a conditioned reflex, whereas the same action after the unconditional stimulus was introduced was deemed an unconditioned reflex. Each mouse was subjected to 30 runs per day for a total of 5 days (150 runs altogether). The above training method was carried out with an automated CTC shuttle-box conditioned reflex tester, and the results recorded.¹

Indices for the behavioral toxicology:

(i) *Strengthened Conditioning Run Number (SCRN)*: This term gives the run number where the first case of three consecutive conditioned reflexes occurred, used here as an indicator of the avoidance learning ability of the mice.

(ii) *Conditioned Reflex Rate (CR%)*: This figure is the percentage of conditioned reflexes occurring in each daily session (1-30, 31-60, 61-90, 91-120, and 121-150).

(iii) *Total Time (TT)*: the total amount of time required to complete all 150 of the runs, in seconds.

(iv) *Conditioned Lag (CL)*: This is the amount of time, in seconds, between the appearance of the conditional stimulus and when the mouse passes into the adjoining room, averaged over the 150 runs.

(v) *Conditioned Reflex (CR)*: This is the number of conditioned reflexes during the 150 trials.

Determination of the cholinesterase activity in brain tissue: Cholinesterase activity was determined by the acetylthiocholine and 5,5'-dithio-bis(2-nitrobenzoic) acid (DBNB) method.²

Statistical methods: The results of the testing were analyzed using F and t testing.

RESULTS

Behavioral toxicology experiment: For each of the high fluoride groups the three avoidance learning indices, SCRN, TT, and CR, showed differences as compared to the control group, and this result was highly significant (the F values were 14.512, 11.515, and 20.403, respectively, $p < 0.01$ in each case). The SCRN and CR were each lower, and the TT longer than the control. In addition, the learning ability decreased with the increasing fluoride in the drinking water. The CL values of each high fluoride group showed no significant difference when compared with the control, $F = 2.045$, $p > 0.05$ (Table 1). The CR% of each high fluoride group in each 30-run session was also markedly lower than the control ($p < 0.01$, $p < 0.05$).

The general learning tendency of all four groups was the same, however; each reached a learning plateau between the 90th and the 150th runs (Table 2).

Table 1. Comparison of conditioned avoidance learning indices (mean±SD)

Group	n	SCRN (Run no.)	TT (s)	CR (No.)	CL (s)
Control	12	65±27	456.7±54.9	56±15	1.85±0.10
HiF I ^a	12	130±22*	558.1±48.4*	22±9*	2.03±0.26
HiF II ^b	11	119±28*	545.5±41.4*	23±13*	1.93±0.22
HiF III ^c	10	94±27 ^{††}	536.0±14.4*	33±8 [†]	1.99±0.07

^aHiF I = 100 mg F/L; ^bHiF II = 50 mg F/L; ^cHiF III = 10 mg F/L. *p<0.01 compared with control, [†]p<0.05 compared with control, ^{††}p<0.05 as compared with HiF I.

Table 2. Comparison of conditioned reflex in different phases (mean±SD)

Group	n	No. 1-30	No. 31-60	No. 61-90	No. 91-120	No. 121-150
Control	12	10.6±7.2	29.4±14.8	36.7±13.7	47.8±12.9	55.4±17.6
HiF I ^a	12	1.9±3.3*	6.9±5.8*	15.3±5.6*	21.1±9.0*	26.7±12.6*
HiF II ^b	11	2.1±2.7*	7.9±7.9*	14.2±11.4*	24.8±13.1*	27.6±16.4*
HiF III ^c	10	3.3±2.2*	9.0±3.9*	20.6±9.3*	37.7±10.8 [†]	39.3±8.4 [†]

^{a-c}See notes a-c to Table 1. *p<0.01 compared with control, [†]p<0.05 compared with HiF I, ^{††}p<0.05 compared with control and also HiF I.

Cholinesterase activity levels in brain tissue: The ChE activity levels for each of the high fluoride groups demonstrated significant difference as compared to the control ($F = 12.668$, $p < 0.01$). The ChE activity levels of all three high fluoride groups were markedly higher than in the control ($p < 0.01$, $p < 0.05$; see Table 3).

Table 3. Results of ChE testing (mean±SD)

Group	n	ChE (U/g)
Control	6	220.98±13.30
HiF I ^a	6	293.17±19.93*
HiF II ^b	6	251.80±19.99 [†]
HiF III ^c	6	268.00±27.75*

^{a-c}See notes a-c to Table 1. *p<0.01 compared with control, [†]p<0.05 compared with control.

DISCUSSION

Active avoidance learning is a basic, easily observable phenomenon among animals. This study made use of a classical physiology experimental technique and shuttle-box equipment to examine the effects of high fluoride drinking water on the avoidance learning ability of mice. The results indicate that high fluoride has a marked effect on learning ability and memory. The various primary indices SCRN, CR, CR%, and TT all showed the fluoride-exposed mice had mental deficits as compared to the controls, with those deficits increasing in severity as the concentration of fluoride in the drinking water increased.

In order to further examine possible mechanisms behind the effects of fluoride on mouse brain function, the activation level of cholinesterase in the brains of the mice were measured, the results showing that the ChE activation or activity level was significantly higher in the fluoride-poisoned mice. There are relatively few

reports on research relating to the effects of fluoride on ChE. One study reports that fluoride has clear inhibitory effect on ChE,³ and other researchers claim that in cases of mild fluoride poisoning the primary effect of fluoride on ChE is indeed inhibitory, but in more serious cases serum ChE activity increases, spurred by varying degrees of damage to nerve tissue. Other animal studies support the conclusion that high fluoride does lead to increased ChE activity.⁴

The increased ChE activity seen in the present study might be a result of the damage to the brain tissue of the mice caused by fluoride poisoning. Choline in nerves of the central cortex plays a key role in recognition and memory; mice that have naturally low activation of choline acetylase demonstrate poor memory retention. Cholinergic drugs can also slow learning speed,⁵ suggesting that acetylcholine (Ach) is important in promoting learning and memory. The lack of cholinergic nerve transmitters in the central cortex resulting from brain tissue damage caused by high fluoride may be one of the reasons the animals in the high fluoride groups showed poor learning ability.

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