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EFFECT OF FLUORIDE EXPOSURE ON SYNAPTIC STRUCTURE OF BRAIN AREAS RELATED TO LEARNING-MEMORY IN MICE

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SUMMARY: Learning-memory behavior was tested in mice on a Y-maze after they drank water containing different concentrations of sodium fluoride. Impairment of the structure of the Gray I synaptic interface in the CA3 area of the hippocampus was analyzed quantitatively by electron microscopy and a computer imaging processor. The main results were: the learning ability of mice drinking water with a high concentration of sodium fluoride showed considerable deterioration, the thickness of post-synaptic density (PSD) was decreased, and the width of the synaptic cleft was markedly increased. The results suggest that impairment of learning capability of mice induced by fluorosis may be closely associated with pathological changes of synaptic structure in the brain.

[Keywords: Brain ultrastructure; Fluoride and mice; Hippocampus; Learning-memory; Synaptic structure.]

INTRODUCTION

Although fluorine is regarded by many as an essential, life-supporting trace element, excessive intake can lead to dental and skeletal fluorosis, with long-term overexpose causing definite harm to the nervous system.[1,2] Chinese public health standards for drinking water mandate a fluoride content of no more than 1.0 mg/L.[3] Nevertheless, fluoride contamination of the Jinhua region in China remains relatively severe, with the water fluoride content in afflicted areas reaching 5 mg/L—enough to merit a label of serious regional fluoride pollution.[4] Given that the hippocampus is an important area of the brain for learning and memory, and that synapses are key neural information transmitters, research on the question of whether the fluoride polluted water from this region is affecting the brain function and brain tissue structure of its inhabitants was undertaken.

Mice were exposed to between 1 and 10 mg NaF/L in their drinking water over a prolonged period to observe effects on their learning and memory resulting from chronic fluoride poisoning. Through examination of the pathological changes in the structures of the synaptic interface within the CA3 area of the hippocampus associated with learning and memory, a potentially new basis for explaining the mechanisms of fluoride intoxication and preventing fluoride poisoning in this region are made available.

MATERIAL AND METHODS

*Laboratory Animals and Subject Grouping:*This study used 28 one-month-old ICR white mice supplied by the Laboratory Animal Breeding Center of the Jinghua Pharmaceutical Testing Facility. They were randomly placed into four groups: the control group (n = 8), the low fluoride group (n = 7), the moderate

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fluoride group (n =7) and the high fluoride group (n = 6), which were respectively given free access to distilled water or 1 mg/L, 5 mg/L, or 10 mg/L aqueous sodium fluoride solution for the ten-week experimental period.

Discrimination learning and memory testing: A three-arm radial maze (i.e., a Y-maze) was used to test discrimination learning and memory.[5] The end of each branch of the response box was equipped with a signaling light. During the experiment, the presence of signaling by the light indicated a safe, non-electrified area, while the other two, unlighted branches were electrified, teaching the mice to discriminate between signals and actively avoid an electric shock. The branch with the light turned on was changed randomly after each run. When the mouse actively moved down the lighted branch in order to avoid electric shock, it was counted as a correct reaction, and any other reaction was an error. The testing was split up into runs of tens, i.e., for each mouse the test was run 10 times, there was then a one-minute break, and then another 10 runs. Nine correct reactions in 10 consecutive runs (90% success rate) was considered the standard for having mastered the test. The number of Y-maze runs each mouse required before it mastered the test was recorded. Lower numbers indicate higher learning ability or quicker leaning speed, and the differences between the various subject groups were compared. Twenty-four hours after mastering the test, each mouse was put through the test ten more times in succession to check the mouse's memory; the number of correct reactions was recorded and expressed as a retained memory percentage.

Brain specimen and testing of synaptic structure attributes: After completion of the behavioral testing phase, two mice from the high fluoride group whose performance in the behavioral tests strongly suggested that they had been affected by fluoride poisoning were humanely killed along with two mice from the control group. A brain sample from each mouse was placed in a 5% glutaric dialdehyde solution and stored in a refrigerator at 4ºC for 24 hrs. Once the brain slices had hardened somewhat, the CA3 area of each hippocampus was extracted by reference to the limbic system diagram.[6] A ultra-thin section was prepared using standard methods, and a JEM-1200EX electron microscope was used to observe and take pictures of the Gray I synapses (see Figure 1).

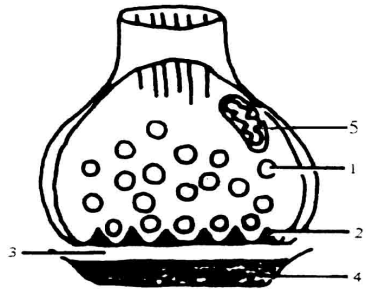

Figure 1. Diagram of a Gray I synapse:
1. Synaptic vesicles
2. Pre-synaptic density projection
3. Synaptic cleft
4. Post-synaptic density (PSD)
5. Mitochondria

Then, according to the methods reported by Luo Lan et al.,[7] a CIA-I computer image analyzer was used do a quantitative analysis of the structures of the synaptic interface, the length of the synaptic active zone, the curvature of the synaptic

interface, the thickness of the post-synaptic density (PSD), and the width of the synaptic cleft.

*Calculation methods:*The double-blind method was used for observations and measurements in this study, and all data is expressed in averages. The data from the learning and memory behavioral testing and from the observation of the synaptic interface of the CA3 area of the hippocampus were respectively subjected to the SSR method and t-test statistical analysis.

RESULTS

Discrimination learning and memory: As Table 1 shows, the number of training runs it took for particular mice to master the task increased along with the fluoride content of their drinking water. The average number of runs needed for the high fluoride group was almost twice that of the control group ($p<0.05$), indicating that high fluoride has a significant negative influence on discrimination learning and memory. Table 1 also shows almost no difference between the retained memory rates of the various subject groups.

Table 1. Effects of fluoride poisoning on discrimination learning and memory

Group	Drinking water sodium fluoride concentration (mg/L)	n	Runs required for mastery	Retained memory after 24 hr (%)
Control	0	7	43.1±7.6	71.8±7.2
Low fluoride	1	7	61.1±10.2	70.0±4.9
Moderate fluoride	5	6	63.2±13.4	70.0±8.6
High fluoride	10	6	77.9±10.3*	70.0±5.8

*p<0.05 compared with the control.

Effect of fluoride on the structure of the synaptic interface of the CA3 area of the hippocampus: See Figures 2–5 for the electron microscopy of the CA3 area of the hippocampus for the high fluoride and control groups.

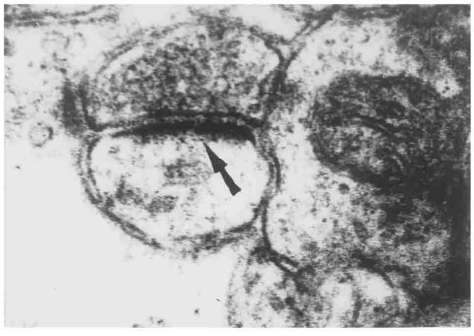

Figure 2. Structure of the synaptic interface in the CA3 area of the hippocampus: control group (×50,000).

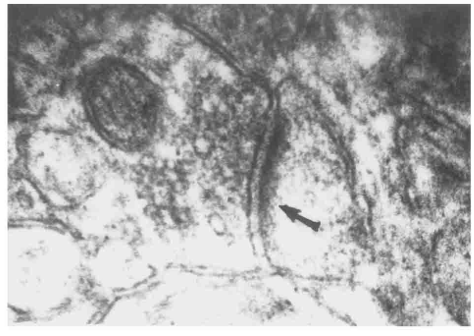

Figure 3. Structure of the synaptic interface in the CA3 area of the hippocampus: high fluoride group (×50,000). The arrow indicates the thin PSD and the wide synaptic cleft

Figures 2 and 3 show that the CA3 area of the hippocampus of both groups have round vesicles inside the pre-synaptic membranes, with thickening of the post-synaptic density and the synaptic clefts that are clearly visible; all of this fits with the specific, characteristic structure of Gray I synapses (i.e., excitatory synapses). In Figure 4 of the hippocampus CA3 area of the control group, the lamellar crista

of the mitochondria inside the pre-synaptic membrane are clearly visible, while in Figure 5 from the high fluoride group, the mitochondrial lamellar crista within the pre-synaptic membrane are indistinct.

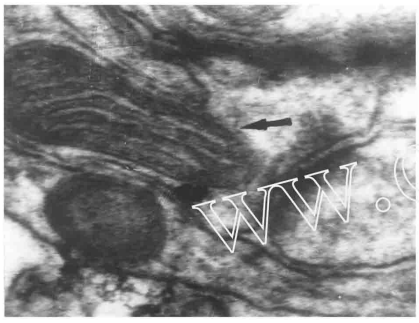

Figure 4. Pre-synaptic mitochondria in the CA3 area of the hippocampus: control group (×50,000). The arrow indicates the clearly-visible mitochondrial lamellar crista.

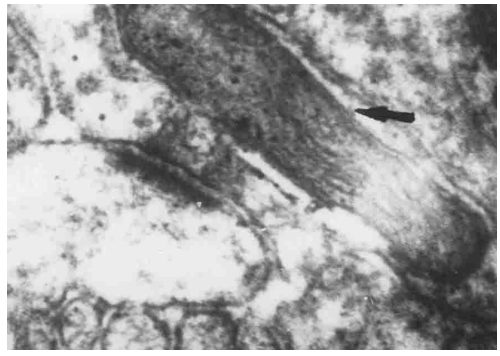

Figure 5. Pre-synaptic mitochondria in the CA3 area of the hippocampus: high fluoride group (×50,000). The arrow indicates the indistinct mitochondrial lamellar crista.

Based on the data from observation of the effects of fluoride poisoning on the synaptic interface of the CA3 area of the mice hippocampuses entered into Table 2, we can see that prolonged intake of increased fluoride has markedly decreased the thickness of the PSD in the affected mice ($p<0.01$), and the synaptic gap is significantly wider ($p<0.01$).

Table 2. Synaptic structure of the CA3 area of the hippocampuses in the mice

Group	Active zone length (nm)	Curvature	Synaptic cleft width (nm)	Post-synaptic density (nm)
Control	315.84±94.77	1.19±0.18	15.68±1.70	55.57±12.61
High fluoride	278.27±102.43	1.17±0.19	17.16±2.45*	39.56±0.97*

*p<0.01 compared with the control.

DISCUSSION

The results of this study indicate that prolonged high fluoride intake can cause definite harm to high level neural activity, i.e., learning in animals. It did not, however, have a significant effect on retained memory, a result that could be due to the short duration of the experimental period, or our results might be consistent with reports[8] of differing neural mechanisms for learning and memory in the brain.Within the brain, the hippocampus is a key region for learning and memory, and the relationship between changes in plasticity of its synaptic structure and learning or remembering behavior has received a great deal of attention from researchers in recent years. Our previous research has already demonstrated that the structure of the synaptic interface is sensitive to change, showing plasticity changes as a result of interference from factors such as age and pharmacology.[5,10] and also that these micro-changes are consistent with macro-changes in learning and memory.

The results of this study show that fluoride poisoning can cause deficits in the learning ability of mice, and can also cause clear pathological changes to the synaptic interfaces within the hippocampus. This suggests that marked effects of chronic fluoride poisoning on learning ability in mice might be related to these pathological changes in the synaptic structure of the brain. Reports in the literature have already demonstrated that fluoride can pass through the blood brain barrier, and that excess fluoride collecting in the brain can cause a reduction in the number of receptors and the synthesis of transmitters in neural cells, leading to retardation of or damage to nerve cell development.[11] Within the PSD there are many kinds of proteins, including receptor proteins. Because of this, fluoride poisoning, which causes a decrease in the number of receptors and the synthesis of transmitters in neural cells, could lead to further pathological changes in the synaptic structure (e.g., widening of the synaptic cleft, decrease in the thickness of the PSD, or abnormalities in the mitochondrial crista, etc.), and these changes to the synaptic interface would necessarily affect the transmission of neural information, ultimately affecting the learning ability and memory of the mice.

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