Int. J. Devl Neuroscience 47 (2015) 165–171

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

# Impact of early developmental fluoride exposure on the peripheral pain sensitivity in mice

Jing Ma[a,1], Fei Liu[a,b,1], Peng Liu[b], Ying-Ying Dong[e], Zheng Chu[b], Tie-Zhou Hou[a,*], Yong-Hui Dang[b,c,d,**]

[a] Affiliated Stomatology Hospital of Xi'an Jiaotong University Health Science Center, Xi'an 710004, Shaanxi, PR China
[b] College of Medicine & Forensics, Xi'an Jiaotong University Health Science Center, Xi'an 710061, Shaanxi, PR China
[c] Key Laboratory of the Health Ministry for Forensic Medicine, Xi'an Jiaotong University Health Science Center, Xi'an 710061, Shaanxi, PR China
[d] Key Laboratory of Environment and Genes Related to Diseases of the Education Ministry, Xi'an Jiaotong University Health Science Center, Xi'an 710061, Shaanxi, PR China
[e] Department of Psychiatry, First Affiliated Hospital of Xi'an Jiaotong, University College of Medicine, Xi'an, Shaanxi, PR China

ARTICLE INFO

*Article history:*
Received 1 June 2015
Received in revised form
19 September 2015
Accepted 21 September 2015
Available online 30 September 2015

*Keywords:*
Fluoride
Developmental exposure
Pain sensitivity
BDNF
Mice

ABSTRACT

Consumption of high concentration of fluoride in the drinking water would cause the fluorosis and chronic pain. Similar pain syndrome appeared in the patients in fluoride therapy of osteoporotic. The aim of the current study was to examine whether exposing immature mice to fluoride would modify the peripheral pain sensitivity or even cause a pain syndrome. We gave developmental fluoride exposure to mice in different concentration (0 mg/L, 50 mg/L and 100 mg/L) and evaluated their basal pain threshold. Von Frey hair test, hot plate test and formalin test were conducted to examine the mechanical, thermal nociceptive threshold and inflammatory pain, respectively. In addition, the expression of hippocampal brain-derived neurotrophic factor (BDNF) was also evaluated by Western blotting. Hyperalgesia in fluoride exposure mice was exhibited in the Von Frey hair test, hot plate test and formalin test. Meanwhile, the expression of BDNF was significantly higher than that of control group. The results suggest that early developmental fluoride exposure may lower the basal pain threshold and be associated with the increasing of BDNF expression in hippocampus.



## 1. Introduction

Fluoride is an element that widely exists in the air, rocks, soil and water, and is added to many articles of daily use, such as toothpastes and mouth rinses. However, excessive consumption of fluoride in the drinking water, which is the most common way that people intake fluoride, could cause chronic fluorosis.

Epidemiological evidences support that fluoride may induce low back pain in people living in the areas with high levels of fluoride in drinking water for more than 20 years. Namkaew and Wiwatanadate (2012) used a retrospective cohort design assessing the dose response of exposure to fluoride-contaminated water to chronic pain in two sub-districts of Chiang Mai, Poo-kha and On-tai, in Thailand. They found that about 348 (65.2%) participants currently had lower back pain; 321 (60.1%) had knee pain, and 198 (37.1%) had leg pain. And this lower back pain was statistically positively related to the average daily fluoride dose of consuming water. Moreover, some researchers also reported the fluoride-specific side effects, the lower-extremity pain syndrome, as the treatment for osteoporosis using the fluoride (Briancon and Meunier, 1981; O'Duffy et al., 1986; Schnitzler and Solomon, 1985).

Accumulating evidence has indicated that not only the skeletal organs, but also developing brain is vulnerable to fluoride. Overmuch fluoride intake could damage the integrity of cerebral vascular and neuron (Varner et al., 1998), resulting in the cognition deficiency (Chioca et al., 2008; Niu et al., 2009). Our previous study showed that the fluoride exposure during development affects both cognition and emotion in mice (Liu et al., 2014). The mice presented the anxiety/depression-like behaviors.

* Corresponding author.
** Corresponding author at: College of Medicine & Forensics, Xi'an Jiaotong University Health Science Center, Yanta Road W. 76#, Xi'an 710061, Shaanxi, PR China. Fax: +86 29 82655116.
*E-mail addresses:* tiezhou@mail.xjtu.edu.cn (T.-Z. Hou), psydyh@mail.xjtu.edu.cn (Y.-H. Dang).
[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.ijdevneu.2015.09.005
0736-5748/

The hippocampus is a component of limbic system and has long been implicated in learning and memory function. Meanwhile, it contributes to the negative affect and approach-avoidance motivation experienced during pain (Duric and McCarson, 2007; McKenna and Melzack, 2001). Microinjection of lidocaine or glutamate receptor antagonists into dorsal hippocampal alleviated formalin-evoked nociceptive behaviors (McKenna and Melzack, 1992, 2001). These mechanisms are not fully understood yet. Previously evidences suggest that some hippocampal neurons respond exclusively to painful stimulation, and after noxious physical stimulation, some anatomical changes occur in dentate gyrus neurons (Dutar et al., 1985; Khanna and Zheng, 1999; Sinclair and Lo, 1986). BDNF meets many criteria to be defined as a neurotransmitter/neuromodulator in nociceptive pathway. In central and peripheral nervous system, BDNF facilitates the survival of certain neuronal population during development. It is also an important modulator of synaptic plasticity (Pezet et al., 2002). Antagonism of BDNF attenuates the second phase of hyperalgesia induced by formalin, indicating that BDNF is involved in some aspects of peripheral inflammation (Kerr et al., 1999; Thompson et al., 1999). BDNF is highly expressed in the limbic system, primarily the amygdala, the hippocampus and the hypothalamus (Malcangio and Lessmann, 2003).

The present study examined whether exposing immature mice to fluoride would modify their peripheral pain sensitivity or even cause a pain syndrome. We evaluated the mechanical, thermal nociceptive threshold and inflammatory pain, using the Von Frey hair test, hot plate test (HPT) and formalin test (FT), respectively. In order to explore the underlying mechanism of fluoride-elicited pain response, we evaluated the expression of BDNF in hippocampus. We also monitored the weight of animals and open field test (OFT) was applied to assess the locomotor activity of mice.

## 2. Materials and methods

### 2.1. Animals

Four week-old C57/BL male mice from the Animal Center of the College of Medicine, Xi'an Jiaotong University were housed (26 cm × 18 cm × 13 cm) under a controlled 12-h/12-h light–dark cycle (lights on at 7:00 A.M.) at a room temperature of 22 ± 1 °C and 55 ± 5% humidity. Four mice were housed in each cage. Mice were given free access to water and food. Experiments were performed after 4 weeks of feeding; they were considered adults at 8 weeks. The overall design scheme for behavioral tests is shown in Fig. 1. Before each test, the animals were placed in the laboratory for 30 min to be acclimated to the test environment. The experimental protocols were approved by the Xi'an Jiaotong University Laboratory Animal Administration Committee. All efforts were made to minimize the number of animals used and their suffering.

### 2.2. Fluoride administration

Based on fluoride concentrations in drinking water, 24 mice were equally and randomly divided into three groups, that is, control group (distilled water, $n = 8$), mid fluoride (50 mg/L NaF, $n = 8$) and high fluoride (100 mg/L NaF, $n = 8$). There were no significant differences in body weight among groups. During the test, the mice were continually treated with their respective NaF concentrations in drinking water. The blinding method was applied in the experiment, that is, the administration of fluoride, operation of behavior test and the analysis of data were conducted by different people individually.

### 2.3. General condition and body weight

The general condition of each mouse, including body weight, teeth, skin and hair, mental state, and responsiveness were measured and recorded every week.

### 2.4. Open filed test (OFT)

The OFT was performed as previously reported (Xing et al., 2010). An apparatus consisted of a square box of 45 cm × 45 cm × 45 cm was used to measure the locomotor activity. Mice were placed in the central square of the field at the beginning of the test and were allowed 60 min of free exploration. A video-computerized tracking system (SMART, Panlab SL, Barcelona, Spain) was used to record the distance of traveling as a measure of locomotor activity.

### 2.5. Von Frey hairs test

Mice were undertaken the Von Frey hairs test from 4-week-old to 8-week-old, 5 times in total. Ten stimuli were made with each of a series of Von Frey hairs (North Coast Medical Inc., Morgan Hill, USA) comprised of the first 11 monofilaments (0.008, 0.02, 0.04, 0.070, 0.16, 0.40, 0.60, 1.0, 1.4, 2.0, and 4.0 g). The test was performed as previously reported by Bourquin et al. (2006). The test was started with filament 0.008 g and if negative responses, the next stiffer monofilament was applied. The monofilament that first evoked a positive response was defined as the threshold and no further monofilaments were applied. The positive response was determined by paw withdrawal occurring twice in the 10 applications. We measured the number of positive withdrawal responses in ascending order for each monofilament of the series. Relative frequency of paw withdrawal, the ratio of positive responses to the total ten times, was calculated as another index to assess the mechanical sensitivity.

### 2.6. Hot plate test (HPT)

The HPT was performed after OFT and repeated in the consecutive 3 days at an ascending temperature of 46 ± 1 °C, 50 ± 1 °C, 54 ± 1 °C. The data of each group was averaged in 3 different days. Mice were placed on the hot plate apparatus (RB-200, Tme Technology, Cheng du, China) to assess their thermal nociceptive threshold. The test was a modified version of the experimental

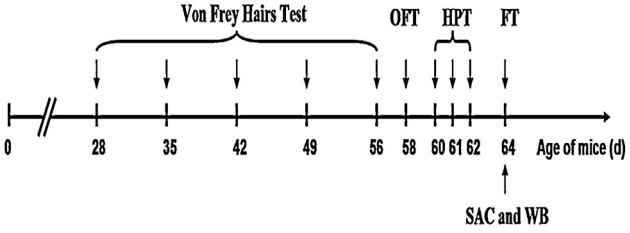


**Fig. 1.** Treatment schedules.
A total of 24 mice were randomly and equally divided into control group and experimental groups, including mid fluoride and high fluoride. In the period of 28–64 days, mice were continually treated with their respective NaF concentrations in drinking water.
Day 28–56: mice were undertaken the Von Frey hairs test every once a week, 5 times in total.
Day 58: the locomotor activities of the mice were measured for 60 min in the OFT after the Von Frey hairs test.
Day 60–62: the HPT was performed after OFT and repeated in the consecutive 3 days at an ascending temperature of 46 ± 1 °C, 50 ± 1 °C, 54 ± 1 °C.
Day 64: mice were received a dorsal surface of the right hind paw subcutaneous injection of 5% formalin with a microinjection. Then the mice were sacrificed and the hippocampus was isolated for Western blotting.